Targeted Expression of SV40 Large T-antigen to Visceral Smooth Muscle Induces Proliferation of Contractile Smooth Muscle Cells and Results in Megacolon*

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Many pathological conditions result from the proliferation and de-differentiation of smooth muscle cells leading to impaired contractility of the muscle. Here we show that targeted expression of SV40 large T-antigen to visceral smooth muscle cells in vivo results in increased smooth muscle cell proliferation without de-differentiation or decreased contractility. These data suggest that the de-differentiation and proliferation of smooth muscle cells, seen in many pathological states, may be independently regulated. In the T-antigen transgenic mice the increased smooth muscle cell proliferation results in thickening of the distal colon. Consequently the distal colon becomes hyper-contractile and impedes the flow of digesta through the colon resulting in enlargement of the colon oral to the obstruction. These transgenic mice thus represent a novel model of megacolon that results from increased smooth muscle cell proliferation rather than altered neuronal innervation.

Under many pathological conditions smooth muscle cells undergo phenotypic modulation in which they change from a contractile quiescent phenotype to a synthetic proliferative state. The phenotypic changes observed depend on the severity of the injury and the specific smooth muscle tissue involved (1–8). Smooth muscle cells in adult tissues are normally quiescent, exhibit very low rates of division, and express high levels of contractile proteins and myofilaments. These differentiated, contractile smooth muscle cells are characterized by the presence of unique isoforms of contractile proteins such as smooth muscle α- and γ-actin, myosin heavy chain, caldesmon, calponin, SM22α, and telokin (4, 9–16). In vitro culture of smooth muscle cells, in the presence of high levels of serum, results in a marked decrease in the expression of many smooth muscle contractile proteins, including smooth muscle myosin, smooth muscle actin, h-caldesmon, calponin, smooth muscle myosin light chain kinase, and telokin with a concomitant increase in the expression of non-muscle contractile protein isoforms (2, 14, 17–19). A similar increase in nonmuscle myosin has been reported in smooth muscle cells during restenosis (20, 21). In contrast, in intimal cells from human coronary arteries, it has been reported that there is reduced expression of SM2 smooth muscle myosin heavy chain without any detectable increase in non-muscle myosin heavy chain B (5). These examples illustrate that smooth muscle cells can exhibit a plethora of phenotypes ranging from fully differentiated quiescent contractile cells to cells expressing low levels of contractile proteins and proliferating rapidly (22).

In most in vivo models of vascular, airway, and intestinal injury, smooth muscle cells are subject to mechanical and/or cytological trauma resulting in exposure to growth factors and re-entry into the cell cycle. Together these agents increase smooth muscle cell proliferation and cause phenotypic modulation altering the contractile properties of the muscle (22, 23). As both proliferation and phenotypic modulation occur at the same time, it is difficult to assess the effect of either process alone on the contractility of the muscle. To examine carefully the relationships between smooth muscle cell proliferation, differentiation, and contractility, we have generated a transgenic mouse model in which visceral smooth muscle cells are induced to proliferate in vivo following expression of an SV40 large T-antigen oncogene. SV40 large T-antigen was specifically targeted to smooth muscle cells using the smooth muscle-specific rabbit telokin promoter. 2.4-Kilobase pair and 310-base pair fragments of the rabbit telokin promoter have been shown to direct high levels of transgene expression in smooth muscles of the gut, airways, reproductive and urinary tract, and low levels of expression in vascular smooth muscle (4, 24). In the current study we show that expression of T-antigen in visceral smooth muscle cells induced cell proliferation without significantly altering the contractile properties of the muscle. In this transgenic model, smooth muscle cell proliferation was accompanied by an increase in the non-muscle B myosin heavy chain isoform without any significant down-regulation of smooth muscle contractile protein isoforms. The increased smooth muscle cell proliferation did not significantly affect the contractile properties of the muscle. These findings show that contractile smooth muscle cells can proliferate without down-regulating contractile protein expression. This suggests that the phenotypic modulation normally associated with proliferating smooth muscle cells following vascular or airway injury in vivo or following in vitro culture of smooth muscle cells occurs independently of proliferation.

EXPERIMENTAL PROCEDURES

Transgenic Mouse—The telokin promoter T2.4-SV40 large T-antigen and T310-SV40 large T-antigen mice were described previously (4, 24). Transgenic mice were generated in C3HeB/FeJ inbred embryos by the Indiana University Transgenic Mouse Facility using standard protocols.

Western and Northern Blotting—Western and Northern blots were performed as described previously (4, 25, 26).

Immunohistochemical Staining—Tissues were collected from transgenic mice, cryoprotected in 20% sucrose, phosphate-buffered saline, overnight. Cryoprotected tissues were embedded in tissue-freezing me-
TABLE I

Phenotypes of telokin-T-antigen transgenic mice

| TRANSGENE | WILD TYPE |
|-----------|-----------|
| No. mice  | Phenotype |
| 2         | Died at birth, no overt pathology |
| 1         | Abdominal tumor |
| 3         | Megacolon |
| 1         | Abdominal tumor |

FIG. 1. Telokin-T-antigen transgenic mice develop megacolon.

The phenotypes of founder animals in which smooth muscle-specific expression of SV40 large T-antigen driven by either 2.4-kilobase pair (Telokin 2.4 T-Antigen) or 310-base pair (Telokin 310 T-antigen) fragments of the telokin promoter are shown.

was established. The onset of the intestinal pathology was delayed in the animals from this founder, potentially due to the effect of the transgene integration site on the timing of transgene expression. The transgene integrated into the X chromosome in this line and telokin-T-antigen transgene expression is not detectable until the animals are approximately 4 weeks old. Mice from this transgenic line all develop megacolon after 4–6 months; the phenotype is 100% penetrant for both male and females and persistent through greater than 10 generations. Although the extent of the enlarged portion of the colon is variable from animal to animal, within the line, the greatest enlargement is always seen in the proximal colon closest to the cecum (Fig. 1).

Analysis of hematoxylin- and eosin-stained cross-sections revealed that the enlarged proximal colon of transgenic animals had a much larger diameter than the proximal colons of wild type mice (Fig. 2A). Analysis of cross-sections of the colon revealed that the thickness of the enlarged proximal colon wall was grossly similar in transgenic and wild type animals (Fig. 2B). In contrast, the density of smooth muscle cells in the transgenic colon was significantly higher in transgenic animals as compared with wild type animals.

T-Antigen-induced Megacolon Is Distinct from Other Mouse Genetic Models of Megacolon—In human megacolon resulting from Hirschsprung’s disease and in several mouse models of megacolon, the lack of intramural ganglia in the distal colon results in constriction of this region causing megacolon (27). To determine if a similar disruption of innervation could account for the phenotype of the telokin-T-antigen transgenic mice, enteric ganglia were visualized by NADPH diaphorase staining of tissue sections (Fig. 3). This analysis demonstrated that unlike other mouse models of megacolon the telokin-T-antigen transgenic animals exhibited enteric ganglia in both the proximal and distal colon. No agangliosis was evident in these animals. Similar results were seen when ganglia were visualized by neurofilament staining (data not shown).

Expression of SV40 Large T-antigen in Visceral Smooth Muscle Induces Smooth Muscle Proliferation—Although the wall of the proximal colon was not overtly thickened, the circular and longitudinal muscle layers displayed hypercellularity (Fig. 2B). There was a 4–5-fold greater nuclear density in the muscle layers of transgenic animals as compared with wild type animal, suggesting that the smooth muscle cells within these layers were proliferating. To determine directly if expression of T-antigen in visceral smooth muscle cells induces cell proliferation, animals were labeled with [3H]thymidine for 6 h prior to sacrifice. Analysis of sections obtained from these animals demonstrated that the telokin-T-antigen transgene induced [3H]thymidine incorporation into the nuclei of smooth muscle cells (identified by smooth muscle myosin staining) of the circular, and longitudinal muscle layers throughout the intestinal tract, indicating that these cells were proliferating (Fig. 4). In contrast, wild type animals exhibited very low levels of smooth muscle cell proliferation as evidenced by no [3H]thymidine-positive smooth muscle cells in many sections. Most of the [3H]thymidine-positive nuclei were confined to the epithelial cells in the crypts of the colon of wild type animals (Fig. 4).

Increased Smooth Muscle Cell Proliferation Occurs Prior to the Development of Megacolon—To determine if the increase in smooth muscle cell proliferation observed in the animals with megacolon was a secondary consequence of the pathology, proliferation was examined in 2-month-old transgenic animals prior to the development of the intestinal pathology. Proliferation was determined by tritiated thymidine uptake into the nuclei of smooth muscle cells. This analysis revealed that visceral smooth muscle cells throughout the gastrointestinal tract...
exhibited high rates of proliferation prior to the development of megacolon (Fig. 4). Despite increased rates of smooth muscle cell proliferation in all regions of the gut, no major histological changes were consistently observed in any region except for the colon. The amount of smooth muscle cell proliferation in the circular muscle layer of proximal and distal colon of wild type and 2-month-old telokin-T-antigen transgenic animals was quantitated by determining the ratio of $^3$H-positive nuclei to total nuclei in this layer. The muscle layers were identified by counterstaining for smooth muscle myosin. This analysis demonstrated that in transgenic animals there was a large increase (10–60-fold) in proliferation in the smooth muscle cells in both the proximal and distal colon as compared with wild type animals (Table II). Calculation of the proliferative index obtained from analysis of several sections from four 2-month-old transgenic animals also revealed that there was no significant

**Fig. 2.** Megacolon results in an increase in the circumference of the colon. Hematoxylin- and eosin-stained cross-sections through the proximal colon of wild type and telokin-T-antigen transgenic animals are shown. A, low magnification. B, high magnification showing the position of the longitudinal muscle (LM), circular muscle (CM), submucosa (SM), muscularia mucosa (MM), and epithelial cells of the mucosa (M).

**Fig. 3.** Colon of telokin-T-antigen transgenic mice contain enteric ganglia. Cross-sections of distal and proximal colon from wild type (WT) and telokin-T-antigen transgenic (TG) animals stained for NADPH diaphorase activity. Positive nerves are stained purple. Several myenteric ganglia are indicated by the arrows. Scale bar indicates 50 μm.
Fig. 4. Visceral smooth muscle targeted expression of SV40 large T-antigen induces smooth muscle cell proliferation. Animals were labeled with tritiated thymidine for 6 h prior to sacrifice. The digestive tract was dissected from the animals, and segments were processed for immunofluorescent analysis as described under “Experimental Procedures.” Cross-sections of gut were stained with antibodies specific for either smooth muscle myosin (SM2) or T-antigen (TAG), both of which are only expressed in smooth muscle cells. Some sections were also stained with antibodies to non-muscle myosin heavy chain B (NMHC-B; a generous gift from Dr. Robert Wysolmerski) as indicated. Stained sections were then dipped in photographic emulsion to identify nuclei that had incorporated triitated thymidine. Staining was visualized under epifluorescence with additional back light illumination to visualize both the antibody staining (green) and the triitated thymidine-positive nuclei (black granules). Sections shown are from distal colon (DISTAL), proximal colon (PROX), ileum, jejunum (JEJ), and stomach, as indicated. Scale bars represent 50 μm.

Identification of the Physiological Defect Resulting in Megacolon—In all known models of megacolon the pathological enlargement of the colon occurs subsequent to blockage of the distal colon and reflects the stretch imposed on the wall of the colon by the continued movement of digesta into the obstructed region. There is no obvious physical obstruction in the colon of telokin-T-antigen transgenic animals, suggesting that, similar to other animal models of megacolon, it is likely that altered contractility results in the obstruction. However, in contrast to other mouse models of megacolon, the impaired contractility is not a result of agangliosis of the distal colon (Fig. 3). To investigate possible mechanisms that cause the megacolon in the telokin-T-antigen transgenic animals, transgenic mice were examined at 2 months of age prior to the development of any gross intestinal pathology. Two possible models can be envisaged by which the contractility of the colon could be altered such that digesta accumulates in the proximal colon. Either the contractility of the distal colon is increased resulting in a constriction that prevents the movement of digesta out of the proximal colon or the contractility of the proximal colon could be decreased such that it is unable to move digesta to the distal colon. Several physiological parameters were examined in order to identify the cause of the motility defect that results in the obstruction of the colon.

Maximal Contractile Responses of Colonic Muscle Rings Are Not Significantly Different between Transgenic and Wild Type Mice—To determine the effect of increased smooth muscle cell proliferation on the physiological function of the muscle, colonic muscle rings were isolated and their contractile properties examined. Three rings of proximal and 3 rings of distal colon were analyzed from each of 6 animals. Maximal contractions in response to KCl (80 mM), electrical (20 Hz, 80 V), and carbachol (10⁻⁶ M) stimulation, normalized to the cross-sectional area of the circular muscle layer in each sample, were not significantly different between wild type and transgenic animals (Fig. 5, A–C). In contrast, both wild type and transgenic distal colon exhibited higher maximal contractions to electric and carbachol stimulation as compared with proximal colon (p < 0.05; Fig. 5, B and C).

The contractile responses to carbachol were further evaluated to determine if the sensitivity to agonist stimulation was altered in the transgenic animals. In both proximal and distal colon the carbachol dose-response curves of transgenic animals were shifted to the right as compared with wild type animals, indicating that muscle from transgenic animals was less sensitive to agonists compared with muscle from wild type animals (Fig. 5, E and F). The concentration of carbachol required to produce a half-maximal contraction increased from 4.4 × 10⁻⁷ to 9 × 10⁻⁷ M in distal colon and from 8 × 10⁻⁷ to 1.7 × 10⁻⁶ M in proximal colon.

The Circular Muscle Layer in the Distal Colon of Telokin-T-
The thickness of the circular muscle layer of the proximal and distal colon of 2-month-old transgenic and wild type mice was determined by morphological analysis of hematoxylin- and eosin-stained sections of the colon, using Metamorph Image analysis software (Fig. 5D). Results from this analysis demonstrated that in both wild type and transgenic animals the circular muscle layer in the proximal colon is significantly thicker than in the distal colon ($p < 0.01$, Fig. 5D). In addition, the circular muscle layer of the distal colon of transgenic animals was significantly thicker than the wild type animals ($p < 0.01$). In contrast, there was no significant difference in the proximal colon between transgenic and wild type animals.

The Distal Colon from Telokin-T-antigen Transgenic Animals Is More Contractile per Unit Length Than Distal Colon from Wild Type Animals—The data shown in Fig. 5 demonstrate that the maximal contractile responses of transgenic and wild type colon are similar when normalized to the cross-sectional area of circular muscle. However, the circular muscle in the distal colon of transgenic mice was significantly thicker than in wild type animals (Fig. 5D). Together these data suggest that per unit length the transgenic distal colon is more contractile than wild type. When the maximal forces obtained from colonic rings are expressed per unit length, it is apparent that although there is little change in the contractility of wild type and transgenic proximal colon, the transgenic distal colon is significantly greater than wild type distal colon. In addition, the contractility of wild type proximal colon is greater than the contractility of wild type distal colon (Table III). This pattern is generally reversed in telokin-T-antigen transgenic animals in which the distal colon is more contractile in response to electrical and KCl stimulation than the proximal colon.

### TABLE II

| Proximal       | Distal       |
|----------------|--------------|
| $^3$H/total    | Percent      | $^3$H/total | Percent |
| TG-1           | 89/1280      | 6.95        | 118/1296 | 8.3 |
| TG-2           | 147/1367     | 10.75       | 67/1296  | 5.2 |
| TG-3           | 13/300       | 4.3         | 24/296   | 8 |
| TG-4           | 9/237        | 3.4         | 26/574   | 4.5 |
| TG-Total       | 258/3184     | 6.35 $\pm$ 3.3% | 235/3586  | 6.5 $\pm$ 1.9% |
| WT-1           | 5/1579       | 0.32        | 7/606    | 1.16 |
| WT-2           | 0/350        | $<0.29$     | 3/338    | 0.89 |
| WT-3           | 1/1019       | 0.098       | 0/208    | $<0.48$ |
| WT-4           | 0/83         | $<1.2$      | 5/803    | 0.62 |
| WT-Total       | 6/3031       | $<0.48$     | 15/1955  | $<0.79$ |

T-antigen-induced Smooth Muscle Proliferation Does Not Ef-
Smooth Muscle Cell Proliferation without De-differentiation

Specific, targeted expression of SV40 large T-antigen to visceral smooth muscle cells stimulates the cells to re-enter the cell cycle and results in proliferation of contractile smooth muscle cells, without inducing de-differentiation. This leads to an increase in the contractility of the distal colon and results in the development of megacolon. It is likely that the hypercontractile distal colon creates a restriction to the passage of digesta through the colon, causing the proximal colon, oral to the restriction, to enlarge producing the megacolon phenotype.

Visceral smooth muscle hypertrophy is a common pathology of many human diseases (23). Diseases that result in partial obstruction of the gut, such as Hirschsprung’s disease, cause a marked smooth muscle cell hypertrophy in cells oral to the obstruction, leading to megacolon. Hypertrophy of the gut following partial obstruction is accompanied by a large increase in the circumference of the intestine oral to the obstruction; this results from increased smooth muscle cell proliferation, in addition to cellular hypertrophy (23). Based on ultrastructural investigations it was shown that the proliferating cells maintained a full complement of myofilaments and specialized structures suggesting that they remained fully differentiated. Similarly, hypertrophy of smooth muscle cells in the urinary bladder occurs following partial obstruction of the outflow tract, and hypertrophy of smooth muscle cells in the trachea and bronchi are seen in chronic asthmatic patients. The hypertrophy of the gut in telokin-T-antigen transgenic mice, although superficially similar to the hypertrophy resulting from experimental constriction of the gut, is morphologically distinct in that most of the increase in muscle volume results from

![Diagram](Image)

**FIG. 5.** The contractility of colonic muscle from telokin-T-antigen transgenic mice is similar to wild type. Rings of colon (approximately 4 mm in length) were cut from the proximal and distal colon of 2-month-old telokin-T-antigen transgenic (TG, hatched bars) and wild type (WT, solid bars), age-matched animals. Muscles were hung in a myobath, and their maximal contractile responses to various agonists was measured as described under “Experimental Procedures.” A, contractions were initiated by addition of 80 mM KCl. B, contractions were initiated by electrical field stimulation (80 V, 20 Hz, 10 s). C, contractions were initiated by addition of 10⁻⁴ m carbachol. Contractions, measured in grams, were normalized to the cross-sectional area of the circular muscle layer in each ring (as this is the muscle layer contributing to the contraction). Three distal and three proximal rings were analyzed from each animal. The data presented represents the mean ± S.E. obtained from 6 animals. Data were statistically analyzed by analysis of variance to identify groups that were statistically different from each other. Statistically significant differences (p < 0.05) are indicated on each graph, together with their p values. Data obtained from wild type animals are indicated by solid bars, and data obtained from transgenic animals are shown by hatched bars. D, the thickness of the circular muscle layer of proximal and distal colon was determined by morphometric analysis of hematoxylin- and eosin-stained sections using Metamorph software. Data represent the mean ± S.E. obtained from a total of at least 15 sections obtained from 4 to 6 different animals. Statistical differences were analyzed by analysis of variance; values having a p value <0.05 were considered not significant. Data obtained from wild type animals is shown by solid bars and from transgenic animals by hatched bars. E and F, dose-response of muscle rings to carbachol. Three distal and three proximal rings were analyzed from each animal. The data presented represent the mean ± S.E. data obtained from 3 animals. Data are expressed as a percentage of the maximal carbachol-induced contraction. E, samples obtained from proximal colon. F, samples obtained from distal colon. Closed symbols indicate data from wild type animals and open symbols from transgenic animals, as indicated.

**TABLE III**

| Mean force per unit length | Proximal WT | Proximal TG | Distal WT | Distal TG |
|---------------------------|-------------|-------------|-----------|-----------|
| KCl                       | 1174        | 1048        | 1047      | 1565      |
| Carbachol                 | 1311        | 1166        | 1164      | 2208      |

**DISCUSSION**
extracts were prepared from samples of proximal (A) and distal (B) colon from 2-month-old wild type and telokin-T-antigen transgenic mice \((n = 4)\). Transgenic mice did not display any overt megacolon. 15 \(\mu\)g of each extract were analyzed by Western blotting with specific antibodies to several contractile protein isoforms. Antibodies used were specific for smooth muscle myosin heavy chain SM1 (SM1), smooth muscle myosin heavy chain SM2 (SM2), non-muscle myosin heavy chain A (nmHCA), non-muscle myosin heavy chain B (nmHCB), h- and l-caldesmon (hCALD and l-CALD), 130-kDa smooth muscle myosin light chain kinase (smMLCK), and 220-kDa non-muscle myosin light chain kinase (nmMLCK), telokin, SM22\(\alpha\), and smooth muscle \(\alpha\)-actin. Proteins shown in brackets were not expressed at detected levels in the extracts analyzed. The positions of molecular mass markers are indicated at the left of the blots.

**FIG. 6.** Smooth muscle contractile proteins are not down-regulated in telokin-T-antigen transgenic smooth muscle. Tissue extracts were prepared from samples of proximal (A) and distal (B) colon from 2-month-old wild type and telokin-T-antigen transgenic mice \((n = 4)\). Transgenic mice did not display any overt megacolon. 15 \(\mu\)g of each extract were analyzed by Western blotting with specific antibodies to several contractile protein isoforms. Antibodies used were specific for smooth muscle myosin heavy chain SM1 (SM1), smooth muscle myosin heavy chain SM2 (SM2), non-muscle myosin heavy chain A (nmHCA), non-muscle myosin heavy chain B (nmHCB), h- and l-caldesmon (hCALD and l-CALD), 130-kDa smooth muscle myosin light chain kinase (smMLCK), and 220-kDa non-muscle myosin light chain kinase (nmMLCK), telokin, SM22\(\alpha\), and smooth muscle \(\alpha\)-actin. Proteins shown in brackets were not expressed at detected levels in the extracts analyzed. The positions of molecular mass markers are indicated at the left of the blots.

hyperplasia rather than hypertrophy of smooth muscle cells (Figs. 1 and 2). Although the size of the smooth muscle cells has not been directly determined in the T-antigen transgenic mice, there is a dramatic 4–5-fold increase in the nuclear density of the circular muscle layer, with a relatively small increase in its thickness (less than 2-fold). These physical constraints imply that the smooth muscle cells in the telokin-T-antigen transgenic animals are likely to be smaller than cells from wild type animals. Although the contribution of smooth muscle cell hyperplasia to the phenotype of the muscle in experimentally induced gut hypertrophy is not well understood, several investigators have shown that proliferating smooth muscle cells can be found in the hypertrophic gut (reviewed in Ref. 23). Similar to other experimental and genetic models of gut hypertrophy, the telokin-T-antigen transgenic mice model also results in a large increase in muscle volume without altering the stratified architecture of the gut. This demonstrates the remarkable ability of the intestine to maintain the normal organization of the external muscle, mucosal, or submucosal layers even in the presence of a dramatic increase in smooth muscle cell proliferation directed by a viral oncogene.

In previous animal models of visceral smooth muscle hypertrophy, a partial obstruction of the colon placed a mechanical strain on the wall of the gut triggering the hypertrophic response. In the telokin-T-antigen transgenic animals, mechanical strain also results from a constriction of the colon. The subsequent remodeling and enlargement of the gut is likely to occur as a result of this mechanical strain. Unique to the telokin-T-antigen transgenic animals an increase in smooth muscle cell proliferation is likely to cause the initial constriction. Morphological measurements of the distal colon in transgenic animals show that the circular muscle layer is significantly thicker than in wild type animals. This results in the increased contractility of the distal colon, and it is likely that it is this increased contractility that presents a constriction to the flow of digesta throughout the colon. The increased pressure of the luminal contents on the oral side of the constriction will then provide the mechanical strain needed to induce remodeling and hypertrophy of the gut. Thus, in addition to loss of relaxing innervation of the colon, as seen in aganglionic megacolon, smooth muscle cell hyperplasia leading to muscle hypercontractility can also lead to megacolon. These results suggest that an increase in the rate of smooth muscle cell proliferation in the wall of the gut may play a role in the etiology of intestinal diseases, such as idiopathic megacolon, that result in megacolon without loss of neural innervation (28).

The telokin T-antigen transgenic model provides a novel system in which to study the effects of smooth muscle cell proliferation on the contractile properties of muscle in vivo. In young transgenic animals prior to the intestinal remodeling, smooth muscle cells in the wall of the intestine are proliferating rapidly with a proliferative index of 6% as compared with <0.8% in wild type animals (Table II). Analysis of intestinal contractility in these animals provides a novel system to investigate the effects of increased smooth muscle cell proliferation alone, in the absence of overt mechanical stimuli, on contractility. Results from this analysis clearly show that, although the colonic smooth muscle cells from telokin-T-antigen transgenic animals are proliferating, this does not effect the maximal contractile response of the muscle to agonists. Neither receptor-independent (KCl) nor receptor-dependent (carbachol, electric stimulation) responses were significantly different between wild type and transgenic animals (Fig. 5). The only difference observed was a small rightward shift in the dose-response curve to carbachol in the transgenic animals (Fig. 5, E and F). Together with these physiological findings, biochemical
analysis of contractile protein expression revealed that there was no significant down-regulation of contractile protein expression in the proliferating colon (Fig. 6). These findings are consistent with previous studies on the lethal spotting mouse model of megacolon, in which no decrease in contractile protein expression was observed in the hypertrophic colon (29). The most significant change in contractile protein expression observed in telokin-T-antigen transgenic animals was an increase in the non-muscle B myosin heavy chain isoform. In contrast, no increase in the expression of the non-muscle or embryonic isoforms of l-caldesmon, myosin light chain kinase, or non-muscle myosin heavy chain A was observed. This suggests that the proliferating muscle has not simply reverted to an earlier embryonic state. A similar increase in non-muscle myosin B isoforms of l-caldesmon, myosin light chain kinase, or non-muscle myosin heavy chain was observed. This suggests that the proliferating muscle is not obligatorily linked to its state of differentiation.

Results presented provide direct evidence that visceral smooth muscle cells can proliferate in vivo without down-regulating contractile protein expression or without affecting the contractile properties of the muscle. These results imply that the phenotypic modulation of smooth muscle cells that occurs following vascular or airway injury or when cells are cultured in vitro is likely to be regulated independently of changes in cell proliferation. In support of this concept, it has been shown that protein kinase G promotes differentiation of cultured vascular smooth muscle cells without altering their ability to proliferate (33). These observations support a growing body of evidence suggesting that the proliferative potential of a vascular or visceral smooth muscle cell is not obligatorily linked to its state of differentiation.

REFERENCES

1. Majesky, M. W., Giachelli, C. M., Reidy, M. A., and Schwartz, S. M. (1992) Circ. Res. 71, 759–768
2. Ueki, N., Sobue, K., Kanda, K., Hada, T., and Higashino, K. (1987) J. Cell Biol. 104, 1535–1542
3. Glukhova, M. A., Sivak, P. N., Kuro-o, M., Kimura, K., Nakahara, K., Takewaki, S.-I., Ueda, M., Yamaguchi, H., Yokogawa, K., and Periasamy, M. (1993) Circ. Res. 73, 1002–1012
4. Chauncey, A., Scatena, M., Faccio, M., Ferraro, P., Paletto, P., Passerini-Giostri, C., Pagano, F., and Sartore, S. (1993) Exp. Cell. Res. 207, 310–320
5. Kim, Y. S., Wang, Z., Levin, R. M., and Chacko, S. (1994) Mol. Cell. Biochem. 131, 115–24
6. Malmoqvist, U., Arner, A., and Uvelius, B. (1991) Am. J. Physiol. 260, C1085–C1093
7. Li, L., Miano, J. M., Cserjesi, P., and Olson, E. N. (1994) Circ. Res. 78, 188–195
8. McHugh, K. M., Crawford, K., and Lessard, J. L. (1991) Dev. Biol. 148, 448–458
9. Miano, J. M., Cserjesi, P., Ligon, K. L., Periasamy, M., and Olson, E. N. (1994) Circ. Res. 75, 805–812
10. Miano, J. M., and Olson, E. N. (1994) J. Biol. Chem. 271, 7095–7103
11. Nagai, R., Kuro-o, M., Babij, P., and Periasamy, M. (1989) J. Biol. Chem. 264, 9734–9737
12. Pinna, M., Herzog, M., Vandekerckhove, J., and Smirnov, V. N. (1992) FEBS Lett. 274, 159–162
13. Sobue, K., and Sellers, J. R. (1991) J. Biol. Chem. 266, 12115–12118
14. Haeberle, J. R., Hathaway, D. R., and Wilcox, C. L. (1992) J. Muscle Res. Cell Motil. 13, 81–89
15. Gabbiani, G., Kocher, O., Bloom, W. S., Vandekerckhove, J., and Weber, K. (1984) J. Clin. Invest. 73, 148–152
16. Halayko, A. J., Salari, H. M. A. X., and Stephens, N. L. (1996) Am. J. Physiol. 270, L1040–L1051
17. Rovner, A. S., Murphy, R. A., and Owens, G. K. (1986) J. Biol. Chem. 261, 14740–14745
18. Degen, H., Scott, N. A., Martin, F., Simonet, L., Bernstein, K. E., and Wilcox, J. N. (1997) Circ. Res. 80, 514–519
19. Leclerc, G., Isner, J. M., Kearney, M., Simon, M., Safran, R. D., Baim, D. S., and Weir, L. (1992) Circulation 85, 1583–1593
20. Owens, G. K. (1995) Physiol. Rev. 75, 487–517
21. Gabella, G. (1990) Anat. Embryol. 182, 409–424
22. Smith, A. F., Bigsby, R. M., Word, R. A., and Herring, B. P. (1998) Am. J. Physiol. 274, C1188–C1195
23. Gallagh, P. J., and Herring, B. P. (1991) J. Biol. Chem. 266, 23945–23952
24. Gallagh, P. J., Herring, B. P., Griffin, S. A., and Stull, J. T. (1991) J. Biol. Chem. 266, 23936–23944
25. Smith, A. F., Bigsby, R. M., Word, R. A., and Herring, B. P. (1998) Am. J. Physiol. 274, 1188–1195
26. Wartiovaara, K., Salo, M., and Sariola, H. (1994) Ann. Med. 26, 66–74
27. Gattuso, J. M., Kam, M. A., and Talbot, J. C. (1997) Gut 41, 252–257
28. Siegman, M. J., Butler, T. M., Moers, S. U., Trinkle-Mulcahy, L., Narayan, S., Adam, L., Chacko, S., Hase, H., and Morano, I. (1997) Am. J. Physiol. 273, C1656–C1665
29. Sartore, S., Chiavegato, A., Franch, R., Faggini, E., and Pauletto, P. (1997) Arterioscler. Thromb. 17, 1210–1215
30. Woodcock-Mitchell, J., White, S., Stirewalt, W., Periasamy, M., Mitchell, J., and Low, R. B. (1993) Am. J. Respir. Cell. Mol. Biol. 8, 617–625
31. Simon, M., and Rosenberg, R. D. (1992) Circ. Res. 70, 835–843
32. Boerth, N. J., Dey, N. B., Cornwell, T. L., and Lincoln, T. M. (1997) J. Vasc. Res. 34, 245–259