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Selective Regulation of MMP and TIMP mRNA Levels in Tree Shrew Sclera during Minus Lens Compensation and Recovery

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Abstract

Purpose—In juvenile tree shrews, a minus-power lens placed in front of the eye produces increased axial elongation and a myopic shift in refractive state that compensates for the power of the lens. Scleral tissue remodeling and modulation of the mechanical properties of the sclera occur during lens compensation. In this study, the time course of changes in scleral mRNA levels of three MMPs and three TIMPs during compensation for a minus lens and during recovery was investigated, to determine which, if any, are temporally associated with changes in the mechanical properties of the sclera and the axial elongation rate.

Methods—Competitive RT-PCR was used to measure the levels of mRNA for MT1-MMP, MMP-2, MMP-3, TIMP-1, TIMP-2, and TIMP-3 in the scleras of tree shrews that had received either 1, 2, 4, or 11 days of monocular −5-D lens treatment, or 11 days of −5-D lens treatment followed by 2 or 4 days of recovery.

Results—Relative to their control eyes, treated eye MT1-MMP and MMP-2 mRNA levels were significantly higher, and TIMP-3 levels were lower by 1 to 4 days of minus lens treatment. These differential effects were absent by 11 days of treatment when the treated eyes had compensated for the lens. The levels of all three TIMPs spiked upward in both eyes after 2 days of recovery. The differential changes in MT1-MMP, MMP-2, and TIMP-3 mRNA levels were all restricted to the treated eye and were temporally associated with the differential changes in axial elongation, refractive state, and the previously measured changes in creep rate.

Conclusions—The observed changes in MT1-MMP, MMP-2, TIMP-2, and TIMP-3 mRNA are consistent with visually modulated MT1-MMP activation of MMP-2 and with MT1-MMP degradation of scleral extracellular matrix components. These data constitute further evidence that visual signals modulate gene expression of selected MMPs and TIMPs to control scleral remodeling, the mechanical properties of the sclera, axial elongation, and refractive state.

Previous studies have provided evidence that a visually guided emmetropization mechanism adjusts the growth of the eye to reduce any mismatch between the eye's axial length and optical power.¹⁻⁴ Experimental manipulation of refractive state in animal models provides particularly strong evidence for the existence of this mechanism. For example, the eyes of avians⁵⁻⁸ and mammals⁹⁻¹³ can be induced to increase their axial elongation rate within days or weeks by placing a minus-power lens or diffuser in front of the eye. In response to a minus lens, the eye elongates until the increase in vitreous chamber depth “compensates” for the decrease in optical power and then adjusts its elongation rate to maintain the match. When the minus lens is removed, the eye is myopic and responds by slowing its axial elongation rate producing refractive recovery.

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Manipulations of the visual environment that trigger a response from the emmetropization mechanism also induce tissue remodeling in the sclera.¹⁴⁻¹⁹ In tree shrews, which, like other eutherian mammals, have an all-fibrous sclera,²⁰ the tissue remodeling does not appear to modulate growth per se, but rather alters the mechanical properties of the sclera and, as a consequence, alters the ability of the sclera to resist the relatively low but constant expansive force of intraocular pressure.^{21,22} In a previous study,²¹ it was found that changes in creep rate (a quantitative measure of viscoelasticity) were closely associated with changes in axial elongation rate during minus-lens treatment (creep rate higher) and recovery (creep rate lower), suggesting that modulation of the mechanical properties of the sclera may control axial elongation. Precisely controlled scleral tissue remodeling is probably responsible for the changes in the mechanical properties of the sclera.

Tissue remodeling is a complex process that involves both synthesis and degradation of the extracellular matrix (ECM). Numerous proteins are involved, including structural components such as collagen and proteoglycans,²³ enzymes such as the matrix metalloproteinases (MMPs) that degrade ECM proteins,²⁴ and tissue inhibitors of metalloproteinases (TIMPs) which bind to and inhibit the activity of the MMPs. In tree shrews, scleral remodeling in eyes developing induced myopia is characterized by decreased levels of collagen,^{16,25} decreased levels of sulfated and unsulfated glycosaminoglycans (GAGs)^{16,19} (Norton TT et al. *IOVS* 1998;39:ARVO Abstract 2312; German A et al. *IOVS* 1999;40:ARVO Abstract 2387), and increased levels of gelatinase-A (MMP-2).²⁶ In a previous study in tree shrews that involved monocular form deprivation (MD) to induce myopia,²⁷ we found that mRNA levels for MMP-2, MMP-3, collagen 1(I), and TIMP-1 were altered during form deprivation and recovery, suggesting that modulation of scleral gene expression may control scleral tissue remodeling and scleral extensibility. When MD is used to induce myopia in one eye, the axial elongation rate and scleral creep rate increase and remain elevated. When minus-power lenses are used, axial elongation and scleral extensibility rise at first. Then, as the axial length reaches its new target, they return to normal.²¹ If the pattern of changes in mRNA levels is causally related to the tissue remodeling and scleral extensibility, then inducing myopia with a minus-power lens should cause changes in mRNA levels for the same proteins as occurs when MD is used to induce myopia. However, the time course of the changes should differ, in keeping with the different time course of the changes in scleral extensibility and axial elongation rate.

In the present study, we focused on the mRNA levels of MMPs and TIMPs that data suggest are involved in the remodeling of fibrous tissue such as sclera. For example, MMP-2 appears to be involved in scleral remodeling,²⁶⁻²⁸ and an important mechanism for the activation of pro-MMP-2 is thought to be cell surface activation by MT1-MMP,^{29,30} a mechanism that also involves TIMP-2 and possibly TIMP-3. Activation of MMP-2 through this mechanism is dependent on the relative levels of these molecules, and appropriate changes in their mRNA levels can suggest that this mechanism is active. MT1-MMP may also directly degrade scleral ECM components. Fibroblasts from MT1-MMP deficient mice have virtually no degradative effect on type I collagen,³¹ suggesting that MT1-MMP may be critical in producing the loss of collagen that occurs during the development of experimental myopia in tree shrews.^{16,25}

Methods

Experimental Groups and Goggle Procedure

Ten groups of tree shrews (*Tupaia glis belangeri*), with five animals in each group, were included in the study. Four monocular -5-D lens groups received 1, 2, 4, or 11 days of lens treatment; two -5-D/recovery groups recovered without the -5-D lens for 2 or 4 days after 11 days of -5-D lens treatment; and four normal groups were measured at 24, 28, 35, or 39 days (± 1 day) after natural eyelid opening (days of visual experience [VE]).

A lightweight goggle frame that clipped onto a dental acrylic pedestal was used to hold the -5-D lens (PMMA, 12-mm diameter, 7.5-mm base curve; Conforma Contact Lenses, Norfolk, VA) in front of the eye.³² The pedestal was installed at 21 ± 1 days of VE, and the animals were allowed 3 days to recover from the minor surgical procedure before visual treatment was begun. The -5-D lens was randomly placed over either the right or left eye. The fellow control eye viewed through the open goggle frame with no lens. Visual treatment was initiated by clipping the goggle frame onto the pedestal, and recovery was initiated by removing the goggle frame. The lens was cleaned twice each day at approximately 9:00 AM and 4:30 PM, and badly scratched lenses were replaced as needed while the animal was placed in darkness (<30 minutes). To control for any systemic effect from the minor surgical procedure to install the pedestal, the animals in the 24, 28, and 35 days of VE normal groups also received a pedestal at 21 ± 1 days of VE, but did not wear a goggle frame.

Ocular Measurements and Tissue Collection

Ocular measurements included measures of refractive state and axial ocular component dimensions. Refractive state was measured in awake animals with an autorefractor (Nidek, Gamagori, Japan),³³ with no ophthalmic or systemic atropine sulfate given at any time due to concerns that atropine could alter the effect of minus-lens treatment in tree shrews.³⁴ Axial component dimensions were measured using A-scan ultrasonography, as previously described.³⁵ Because a primary interest of this study was to relate any changes in mRNA levels to changes in the sclera, the measurements of vitreous chamber depth were made to the anterior surface of the sclera,³⁶ not the vitreo-retinal interface which could reflect a change in choroidal thickness without a change in the location of the anterior surface of the sclera.³⁷ A-scan measures were made at 21 days of VE when the pedestal was installed. Refractive state and A-scan measures were made at the end of the treatment period in the -5-D lens groups. In the -5-D/recovery groups, refractive state only was measured at the end of lens treatment, because A-scan measures required anesthetizing the animal, which may have affected recovery. Both refractive state and A-scan measures were made after recovery. At the end of treatment, all animals received an overdose of pentobarbital sodium, and eyes were enucleated between 10:00 and 11:30 AM. Whole eyes were immediately submerged at room temperature in a stabilization reagent (RNALater; Ambion, Austin, TX) that prevents RNA degradation. Scleras were quickly cleaned of non-scleral tissue at room temperature in the reagent, frozen in liquid nitrogen, and kept at -80°C until RNA was extracted. All procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the Association for Assessment and Accreditation of Laboratory and Animal Care (AAALAC) regulations for the use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Competitive RT-PCR

Competitive reverse transcription-polymerase chain reaction (RT-PCR) was used to measure the levels of mRNA for MT1-MMP, MMP-2, MMP-3, TIMP-1, TIMP-2, and TIMP-3. In competitive RT-PCR a synthetic RNA competitor molecule is used that is added to the RT-PCR reaction in known copy numbers. To maximize the similarity of the amplification efficiencies of the synthetic competitor and the native template, the RNA competitor has the same sequence as the target mRNA and is reverse transcribed and amplified by the same primers used to reverse transcribe and amplify the native mRNA. However, the RNA competitor has an internal deletion (~10%) that allows its PCR product to be distinguished from the native product by size on an electrophoretic gel. When the densities of the native product and the competitor product are equal in a gel, the number of copies of native mRNA in the RT-PCR reaction is approximately equal to the number of copies of competitor added.

New batches of competitors were produced for the present study. Compared with a previous study²⁷ in which different batches of competitors were used, the new competitors produced similar 18s copy numbers, whereas the copy numbers for MMP-2, MMP-3, and TIMP-1 were 10- to 50-fold lower. We believe that this variation is primarily due to a difference in the procedure used to manufacture the competitor. In the previous study, the competitors for MMP-2, MMP-3, and TIMP-1 were cleaned up after the transcription reaction with an RNA extraction kit (SV Total RNA Isolation System; Promega, Madison, WI), whereas the competitor for decorin, collagen, and 18s were gel purified. In the present study all the competitors were gel purified. Gel purification helps ensure that most of the competitor is full length whereas cleanup with the RNA extraction kit could allow some incomplete product to be included. The presence of incomplete competitor molecules would cause an overestimate of the copy number, which is consistent with the higher copy numbers in the previous study. Comparison of the copy numbers obtained with different batches of gelpurified competitors suggests much lower variation of approximately two- to threefold, which may be largely due to the very low spectrophotometer readings used to quantify the competitor.

All measurements in the present study were made with the same batches of competitors. Therefore, relative comparisons within the study between treated eyes, control eyes, and normal eyes, as well as longitudinal comparisons are valid quantitative comparisons. However, the copy numbers reported are an estimate of the true copy number, which is somewhat dependent on the batch of competitor. Therefore, no particular significance should be assigned to the absolute copy number and direct comparisons of copy number with our previous studies^{27,38} that used a different batch of competitor should not be made.

Details of the following procedures have been described previously.³⁸ A brief account follows.

RNA Isolation—Total RNA was extracted from individual whole scleras (SV Total RNA Isolation System; Promega). RNA concentration and purity were determined by spectrophotometry at 260 and 280 nm.

Primers Design—Tree shrew-specific primers were designed for the mRNAs of MT1-MMP, MMP-2, MMP-3, TIMP-1, TIMP-2, TIMP-3, and 18s ribosomal RNA. Sequencing verified the identities of the PCR products. The primer sequences are given in Table 1.

Competitor RNA Construction—Each competitor RNA (cRNA) for this study was made with a kit (Competitor Construction Kit; Ambion) that includes modified nucleotides that render the cRNA molecules RNase resistant.

RT-PCR and Quantification Procedure—A detailed explanation of the RT-PCR and quantification procedure can be found in a previous publication.³⁸ The only modification to the procedure is that two twofold dilutions were performed instead of three.

To compensate for any difference in the quantity or the quality of total RNA in each RT-PCR reaction, the target mRNAs were normalized to the number of copies of 18s rRNA. Results were expressed as copies of target mRNA per copies of 18s. For each sclera, the levels of 18s rRNA and each target mRNA were measured in separate aliquots from the same mastermix by adding the appropriate cRNA and primers.

Statistical Tests

Paired *t*-tests were used to determine whether differences between the treated and fellow control eyes were statistically significant. Analyses of variance (ANOVA) and least significant difference (LSD) post hoc tests were used to test whether values changed significantly over treatment duration and whether differences between normal eyes and the treated or control

eyes were significant. The average value for a normal group was obtained by first averaging the right and left eye data for each animal in the group, and then taking the average of those values for the group. It is not possible to measure creep rate and mRNA levels in the same piece of sclera; therefore, it was not possible to make statistical comparisons between creep rate and mRNA levels in individual animals.

Results

Data from a previous study that measured scleral creep rate during -5 -D lens treatment²¹ are shown in Figure 1 for comparison with the timing of the mRNA changes observed in the present study. The tree shrews in that study received essentially identical -5 -D lens treatment as the animals in the present study and exhibited similar refractive and vitreous chamber depth changes. Creep rate measures were not made after 2 or 4 days of recovery. However, creep rate was dramatically lower in the treated eyes at 2 days of recovery after 11 days of monocular form deprivation.

Refractive State and Ocular Component Dimensions

Treatment with a -5 -D lens produced a myopic shift in refractive state and an increase in vitreous chamber depth in the treated eye, relative to the control eye, that increased with length of treatment (Fig. 2). There was a significant difference in vitreous chamber depth after 1 day and in refractive state after 2 days of treatment (paired t -test, $P < 0.05$). After 4 and 11 days of -5 -D lens wear, there was a significant difference in refractive state and vitreous chamber depth (paired t -test, $P < 0.05$) between the treated and control eyes. After 11 days of lens wear, the refractive difference (-4.5 ± 0.4 D) between the treated and control eyes approximately matched the power of the lens, suggesting full compensation. The animals that were exposed to a 2- or 4-day recovery period had similar amounts of induced myopia (2 days, -4.9 ± 0.2 D; 4 days, -4.8 ± 0.5 D). When recovery was initiated by removing the goggle, the refractive difference and difference in vitreous chamber depth began to diminish. After 2 and 4 days of recovery, the relative myopia and difference in vitreous chamber depth were progressively less than after 11 days of -5 -D, following the pattern of recovery described previously.^{19,21} The refractive state of the control eyes was not significantly different from that of the normal eyes (ANOVA, LSD, $P > 0.05$) at any point during -5 -D lens wear or recovery.⁴⁰⁻⁴² However, the vitreous chamber depth in the control eyes of the 11-day -5 -D group was significantly shorter than in the age-matched 35 days of VE normal group, (Fig. 2C, unpaired t -test, $P < 0.05$), suggesting an effect on the control eyes that was not reflected in the refractive state (Fig. 2A). There were no significant differences in refractive state or ocular component dimensions between the right and left eyes in any of the normal groups.

mRNA Levels

Normal Groups—In the normal animals, the level of a given mRNA was similar in the right and left eyes. The only case where an mRNA level was significantly different was in the 24 days of VE group where the TIMP-3 mRNA level was 18% lower in the right eyes than in the left eyes ($P = 0.034$, two-tailed, paired t -test). There was not a significant difference ($P > 0.05$, ANOVA) in the mRNA level across the four normal groups (24, 28, 35, and 39 days of VE) for any of the six mRNAs studied. Thus, there was no evidence for a normal developmental change in the levels of any of the six mRNAs over the period studied from 24 to 39 days of VE. Because the mRNA levels in the four normal groups (24, 28, 35, and 39 days of VE) were not significantly different for any of the mRNAs, the average normal value for the 20 normal animals in the four normal groups and the 95% confidence interval are shown in Figure 3. This presentation of the normal data provides an estimate of the range of values that would be expected to occur in normally developing animals during this period.

General Patterns—The mRNA levels during -5-D lens treatment and recovery are shown in Figure 3. Similar to our previous study in which we used form deprivation to induce myopia,²⁷ there were two general effects on mRNA levels: (1) differential changes in the levels of some, but not all mRNAs in the treated eyes compared with their untreated fellow control eyes, and (2) binocular changes in both the treated and control eyes compared with the age-matched normal eyes. Statistically significant differential changes in some mRNA levels occurred by 1 or 2 days of lens treatment. The onset of these mRNA level changes occurred at least as early as the changes in refractive state, axial length, and creep rate. The differential effects initially produced by lens treatment were absent by 11 days of treatment when the eyes had finished compensating for the minus lens and in some cases were reversed after 4 days of recovery.

MT1-MMP—MT1-MMP (membrane-type MMP-1) mRNA levels rose rapidly in the treated eyes, reaching a peak at 2 to 4 days of treatment and returned to near normal levels by 11 days of treatment (Fig. 3A). After 2 days of treatment, the level was 55% higher ($P < 0.01$) in the treated eyes than in the control eyes and 104% ($P < 0.001$) higher after 4 days of treatment. The effect on the treated eyes was highly consistent, with all 15 animals in the 1, 2, and 4 day -5-D groups having a higher MT1-MMP mRNA level in the treated eye than in the control eye. The treated eye levels at 2 and 4 days were also significantly higher ($P < 0.05$) than the normal-eye levels. After 11 days of treatment, when the treated eyes had compensated for the -5-D lens and were presumably no longer elongating faster than the control eyes, the MT1-MMP level in the treated eyes was no longer significantly different from the level in the control eyes. There was little, if any, effect on the level in the control eyes at any point during compensation for the lens. During recovery, the MT1-MMP level in the recovering eyes continued to drop back toward normal levels, whereas the level in the control eyes appeared to rise slightly. After 4 days of recovery, the level was 30% higher in the control eyes, but the difference was not statistically significant ($P = 0.14$).

MMP-2—The pattern for MMP-2 (gelatinase-A) mRNA was similar to the pattern for MT1-MMP. During lens treatment, MMP-2 mRNA levels showed an early differential increase in the treated eyes compared with the control eyes (Fig. 3B). After 4 days of treatment, the level was 36% higher ($P < 0.001$) in the treated eyes than in the control eyes. The levels in the treated eyes and the control eyes were lower than in normal eyes after 1 day of treatment, but the difference was not statistically significant, and it is not clear whether the difference is a transient effect on both eyes or is normal variability. After 11 days of lens treatment, the levels in the treated and control eyes were similar. After 2 days of recovery, MMP-2 mRNA levels were not significantly different in the treated and control eyes, and neither was different from normal. After 4 days of recovery (Fig. 3B), the mRNA level in the recovering eyes was 28% lower ($P < 0.02$) than in the control eyes.

MMP-3—There were no statistically significant differential effects on MMP-3 (stromelysin-1) mRNA levels during lens treatment or recovery. However, there was some evidence of a transient binocular spike after 2 days of lens treatment and then again after 2 days of recovery (Fig. 3C). After 2 days of treatment, the mean MMP-3 mRNA level was 166% higher (NS) in the treated eyes and 230% higher ($P < 0.05$) in the control eyes than in the normal eyes. The levels in the treated and control eyes were then similar to normal levels after 4 and 11 days of treatment. After 2 days of recovery, the MMP-3 mRNA level was 204% higher ($P < 0.05$) in the recovering eyes and 225% higher ($P < 0.05$) in the control eyes than in the normal eyes.

TIMP-1—There were no statistically significant differential effects on TIMP-1 mRNA levels during lens treatment or recovery. There appeared to be binocular effects. The TIMP-1 mRNA level in both the treated and control eyes was lower than normal levels after 1, 2, 4, and 11 days of lens treatment (Fig. 3D). The direction of this binocular effect was consistent, but the

difference was not significant for any group (ANOVA, LSD, $P > 0.05$). During recovery, there was a transient binocular increase in the TIMP-1 mRNA level compared with normal eyes after 2 days of recovery (treated eye: 68% higher, $P < 0.05$; control eye: 39% higher, NS), and a return to normal levels in both eyes by 4 days of recovery. The levels in the treated eyes at 2 days of recovery were significantly higher than the those in the treated and control eyes after 1, 2, 4, and 11 days of lens treatment (ANOVA, LSD, $P < 0.05$), whereas the levels in the control eyes at 2 days of recovery were significantly higher than those in the treated and control eyes after 4 days of lens treatment (ANOVA, LSD, $P < 0.05$).

TIMP-2—The TIMP-2 pattern was very similar to the TIMP-1 pattern (Fig. 3E). Like TIMP-1, TIMP-2 showed a binocular trend toward lower levels during lens treatment that was consistent in direction but not quantitatively significant (ANOVA, LSD, $P > 0.05$). Also similar to TIMP-1, there was evidence of a binocular transient spike after 2 days of recovery (recovering eye: 53% higher, NS; control eye: 59% higher, $P < 0.05$) and a return to normal levels by 4 days of recovery. The levels in the treated and control eyes at 2 days of recovery were significantly higher than those in the treated and control eyes after 1, 2, and 4 days of lens treatment (ANOVA, LSD, $P < 0.05$).

TIMP-3—The TIMP-3 mRNA level dropped dramatically in the treated eyes during lens treatment (Fig. 3F). The level in the treated eyes was 54% lower ($P < 0.05$) than in the control eyes after 2 days of treatment and 51% lower after 4 days. The treated eye levels at 2 and 4 days of lens treatment were also significantly lower than the normal level (ANOVA, LSD, $P < 0.05$). Similar to, but in the opposite direction of MT1-MMP, the differential change in the treated eye TIMP-3 mRNA level peaked by 2 days of treatment and was largely gone by 11 days. Also similar to MT1-MMP, the effect was highly consistent with 13 of the 15 animals in the 1-, 2-, and 4- day groups (10 of 10 in the 2- and 4-day groups) having a lower level in the treated eye compared with the control eye. The TIMP-3 pattern during recovery was similar to the TIMP-1 and TIMP-2 patterns with a transient binocular spike upward at 2 days of recovery (recovering eye: 123% higher than normal, $P < 0.01$; control eye: 88% higher than normal, $P < 0.01$) and a return to normal levels after 4 days of recovery. Further, the levels in the treated and control eyes at 2 days of recovery were significantly higher than those in the treated, control, and normal eyes at all other points during lens compensation and recovery (ANOVA, LSD, $P < 0.05$). It is of interest to note that the TIMP-3 mRNA level in the treated eye after 2 days of recovery was four times as high as it was after 2 days of lens treatment.

Discussion

Monocular minus-lens treatment in tree shrews produced significant differential changes in the treated eye scleral mRNA levels of MT1-MMP, MMP-2, and TIMP-3 and appeared to produce binocular changes in the mRNA levels of MMP-3, TIMP-1, and TIMP-2. These changes in the mRNA levels of proteins known to be involved in tissue remodeling extend to minus lenses the findings of an earlier study in tree shrews²⁷ that showed consistent scleral mRNA changes in response to form deprivation and recovery.

Differential Changes

The differential changes in MT1-MMP, MMP-2, and TIMP-3 mRNA levels were all restricted to the treated eye and all appeared to occur at least as early as the differential changes in axial elongation, refractive state, and the previously measured changes in creep rate (Fig. 4).^{21,22} This suggests that these changes in gene expression may be relatively early scleral responses to altered retinal/RPE/choroidal signals arriving at the sclera and that these changes may participate in the tissue-remodeling process that alters the scleral creep rate in the treated eye. There are almost certainly other changes in gene expression that are necessary to produce the

observed tissue remodeling that were not measured in this study. The differential downregulation of the TIMP-3 mRNA level in the treated eye during compensation for the lens is particularly interesting, because a similar change did not occur in the TIMP-1 or -2 mRNA levels. This selective downregulation of only one of the three TIMPs suggests it is not a generalized TIMP effect and further suggests a very specific role for the downregulation of TIMP-3.

Binocular Changes

It is not clear how monocular visual treatment can produce binocular changes in scleral mRNA levels, nor is it clear whether the changes are produced by the visual treatment per se or some other factor. Our previous publication contains a discussion of several possible mechanisms.²⁷ It does not appear that the binocular changes observed in our previous study²⁷ were an artifact of the form deprivation used to induce myopia in that study because minus lenses also produced binocular changes in the present study.

The absence of an obvious effect of the binocular mRNA level changes on the refractive measures in the control eye may appear to be inconsistent with the suggestion that the differential changes may help produce the axial and refractive changes in the treated eye. It should be kept in mind, however, that a change in an mRNA level that occurs in both eyes may not by itself be sufficient to produce an observable change in the control eye, but may, when combined with differential changes, help produce the changes that occur in the treated eye.^{26,43} The concept that a single component of a multicomponent system can be necessary but not sufficient to produce an effect is certainly not novel. In a previous publication, we presented the effects of systemic lathyritic treatment in tree shrews as an example.²⁷ Systemic lathyritic treatment blocks lysyl oxidase-mediated collagen cross-linking in the scleras of both eyes and greatly enhances the axial elongation and myopia produced by form deprivation in the treated eye. There is, however, no axial or refractive effect on the control eye. The lathyritic treatment is necessary to enhance the effect of form deprivation in the treated eye, but is not sufficient to produce an effect in the control eye. Tissue remodeling is a complex process that probably requires the combined expression of numerous genes, and one would not necessarily expect the expression of only one of the genes involved to produce an effect. Although the mechanism and significance of the binocular mRNA level changes is not clear, the absence of a refractive effect on the control eye does not render arguments for the causality of the differential changes invalid.

Role of MT1-MMP in Activation of Pro-MMP-2

The control of pro-MMP-2 activation during the scleral remodeling process is of interest because the amount of activated MMP-2 is known to increase during experimentally induced myopia in tree shrews.²⁶ One mechanism for the activation of pro-MMP-2 is cleavage by MT1-MMP.^{44,45} Although this study did not directly examine pro-MMP-2 activation at the protein level, the observed changes in mRNA levels of MT1-MMP, MMP-2, TIMP-2, and TIMP-3 were generally consistent with the modulation of this mechanism.

MT1-MMP activation of pro-MMP-2 involves, paradoxically, TIMP-2 which is generally associated with suppression of MMP activity and indeed can bind to and inactivate MMP-2.^{30,46-49} TIMP-2 bound to MT1-MMP is thought to form a “receptor” for pro-MMP-2 that presents the pro-MMP-2 molecule to another unoccupied MT1-MMP which cleaves the pro-MMP-2 molecule to initiate the activation process (Fig. 5). Studies suggest that the level of MMP-2 activation via this mechanism is dependent on the relative amount of MT1-MMP, MMP-2, and TIMP-2.^{30,50} The addition of small amounts of TIMP-2 can increase MMP-2 activity, presumably by increasing the available number of MT1-MMP/TIMP-2 “receptors” for pro-MMP-2. However, the addition of more TIMP-2 can then decrease MMP-2 activity,

presumably due to excessive free TIMP-2 that binds to and inhibits MT1-MMP as well as the activated MMP-2.^{30,50} TIMP-1 does not bind MT1-MMP; therefore, excessive TIMP-1 does not affect MT1-MMP activation of proMMP-2.⁴⁵ Depending on the starting levels, appropriate changes in the levels of the three molecules could theoretically lead to an increase in MMP-2 activity. For example, if the relative amount of TIMP-2 compared with the amounts of MT1-MMP and MMP-2 is biased toward inhibition of pro-MMP-2 activation, an increase in MTMMP-1 and MMP-2 along with no change in TIMP-2 could reduce the inhibitory effect of TIMP-2 and increase the activation of pro-MMP-2. This condition is consistent with the results of this study where the levels of MT1-MMP and MMP-2 mRNA increased during lens treatment, whereas TIMP-2 mRNA levels did not increase.

The relative abundance of TIMP-3 may be another factor in the control of pro-MMP-2 activation. TIMP-3, like TIMP-2 binds to and inhibits MT1-MMP⁴⁵ and high levels of TIMP-3 strongly inhibit the activation of proMMP-2.⁴⁵ However, the regulation of TIMP-3 levels specifically targets the activity of MT1-MMP without also affecting the MT1-MMP/TIMP-2 “receptor” for proMMP-2. The downregulation of TIMP-3 mRNA levels during compensation for a minus lens and upregulation during recovery in the present study is consistent with the known increase in activated MMP-2 during experimentally induced myopia and decrease during recovery in tree shrews.²⁶

MT1-MMP's Effects on ECM Components

MT1-MMP may also participate in scleral tissue remodeling by directly cleaving type I collagen and other ECM components. Cleavage of triple helical interstitial collagens into characteristic three-fourth and one-fourth fragments by MT1-MMP has been demonstrated as has cleavage of denatured interstitial collagens, aggrecan, fibronectin, and other ECM molecules.^{51,52} The loss of collagen in the tree shrew sclera during experimentally induced myopia^{16,25} most likely requires the cleavage of native collagen fibrils. Tree shrews, however, like many other species, do not appear to have a true MMP-1 (unpublished observation from this laboratory (1999), based on the inability to amplify MMP-1 mRNA in tree shrew scleral RNA). Although MMP-2 reportedly can cleave native collagen,⁵³ fibroblasts from MT1-MMP-deficient mice completely lack the ability to degrade type I collagen fibrils in vitro,³¹ and the effect is not due to a lack of activation of pro-MMP-2 by MT1-MMP. This suggests that remodeling of the tree shrew sclera, which contains approximately 90% type I collagen, may be critically dependent on direct degradation of collagen by MT1-MMP.

The location of the MT1-MMP molecules on the fibroblast surface, between the collagen lamella, makes it an ideal candidate to cleave collagen at the surface of the lamella, which may form structural elements that retard slippage between the lamella. Such cleavage of collagen at the lamellar surface would be consistent with previous findings that short-term form deprivation produces a loss of type I collagen content²⁵ and a change in the mechanical properties of the sclera^{21,22} but does not appear to alter the collagen fibrils in the interior of the lamella.⁵⁴ (Kang RN et al. *IOVS* 1996;37:ARVO Abstract 1491).

Comparison with Form-Deprivation–Induced mRNA Changes

In the present study, the changes in MMP-2, MMP-3, and TIMP-1 mRNA levels produced by minus lenses were qualitatively similar to the changes produced in these mRNAs by form deprivation in our previous study.²⁷ The MMP-2 patterns were the most similar, with both showing a bidirectional differential effect in the treated eye relative to the control eye: levels were significantly higher in the treated eyes than in the control eyes after 4 days of treatment, and levels were significantly lower in the treated eyes after 4 days of recovery. The MMP-3 pattern was interesting in that it was shifted (relative to the normal levels) compared with the pattern during form deprivation, but was otherwise similar. The TIMP-1 pattern was similar

in that it showed evidence of downregulation in both eyes during treatment and upregulation during recovery. MT1-MMP, TIMP-2, and TIMP-3 mRNA levels were not measured during form deprivation. One difference between form deprivation and minus lens treatment is that all of the differential changes produced by the minus lens in the present study had dissipated at 11 days of lens wear, when the eyes had approximately compensated for the lens. With form deprivation, significant changes persisted in the treated as well as the control eye at 11 days of treatment. The general similarity of the mRNA changes produced by form deprivation and minus lenses is evidence that the two different methods of inducing development of myopia trigger a similar tissue-remodeling process in the sclera with a different time course. Because, with form deprivation the eye does not receive visual feedback to slow its elongation, the scleral changes persist, whereas they dissipate with minus lens wear as the eye completes compensation for the lens. It thus appears that changes induced with a minus lens, though similar to those induced with form deprivation, may be more specifically related to the emmetropization process than those induced with form deprivation. However, taken together, the results of the two studies support the concept of a final common pathway for the control of axial length, regardless of the obvious differences in the visual stimulus and possible differences in the retinal and choroidal processing of the signal.

In conclusion, this study provides evidence for selective regulation of the expression of several key MMPs and TIMPs in the sclera during minus lens compensation and recovery. These mRNA level changes are generally consistent with known tissue-remodeling mechanisms and are temporally associated with the changes in the mechanical properties of the scleral tissue, axial elongation rate, and refractive state. These data provide further evidence that the visually guided emmetropization mechanism modulates scleral gene expression in a consistent, theoretically reasonable manner to fine-tune axial length and the refractive state of the eye.

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Figure 1.

Regulation of the viscoelastic properties of the scleral tissue (measured as creep rate: an increase in length under constant tension) during monocular compensation for a -5-D lens (replotted from Siegwart and Norton.²¹) Creep rate was increased in the treated eyes, relative to control and normal eyes, after 2 days of -5-D lens wear, peaked at 4 days and had decreased by 11 days. Creep rate was measured in 3-mm wide strips of sclera under 3 g of constant tension. In the normal animals, left and right eyes were averaged. No creep rate measures are available for 2 or 4 days of recovery after 11 days of -5-D lens treatment. The creep rates shown at 2 days of recovery are after 11 days of MD. $n = 3$ in each group. Values are mean $\pm$ SEM.

Figure 2.

Ocular refraction and vitreous chamber depth during -5-D lens treatment and recovery. Relative to their fellow control eyes, the treated eyes became increasingly myopic (**A**), and their vitreous chamber became increasingly deep (**C**) as a function of days of -5-D treatment. During recovery, the myopic progression and the increase in vitreous chamber depth abruptly reversed. Values from normal animals (average of left and right eyes) are shown for comparison. (**B**, **D**) The difference between the treated and control eyes. $n = 5$ in each group. Results are expressed as the mean $\pm$ SEM; $*P < 0.05$. Because of the small-eye artifact, a reading of 4 D of hyperopia is estimated to be emmetropia in tree shrews.^{33,39}

Figure 3.

mRNA levels in the treated and control eyes of animals with different durations of -5-D lens or -5-D/recovery and age-matched normal animals. All mRNA levels are expressed as copies per million copies of 18s. (A) MT1-MMP, (B) MMP-2, (C) MMP-3, (D) TIMP-1, (E) TIMP-2, and (F) TIMP-3. Treated and control eye error bars are 1 SEM. The average value and 95% confidence interval are shown for the normal animals. T v C, treated eyes different from control eyes (paired-test, $P < 0.05$); T v N, treated eyes compared with normal eyes (ANOVA, LSD, $P < 0.05$); C v N, control eyes compared with normal eyes (ANOVA, LSD, $P < 0.05$).

Figure 4.

The percentage difference (treated – control eye) in the mRNA levels of the four genes that may be involved in the activation of pro-MMP-2 compared with the percentage difference in creep rate during minus lens compensation and recovery. (A–D) The percentage difference in the average mRNA copy number in the treated and control eyes from Figure 3. As computed, this difference has no associated error bars. *Statistical significance ($P < 0.05$), as shown in Figure 3. (E) The percentage difference in the average creep rate in the treated and control eyes from Figure 1,²¹ in which the animals underwent –5-D lens treatment for similar periods. No creep rate measures are available for 2 or 4 days of recovery after 11 days of –5-D lens treatment. Therefore, the creep rate at 2 days of recovery is after 11 days of MD.

Figure 5.

Schematic showing the suggested relationship between MT1-MMP, TIMP-2, and MMP-2, as it relates to MT1-MMP activation of proMMP-2. MT1-MMP is a membrane-bound MMP that localizes the activation of pro-MMP-2 to the cell surface. TIMP-2 bound to MT1-MMP is thought to form a "receptor" for pro-MMP-2 that presents the proMMP-2 to another MT1-MMP that cleaves the pro-MMP-2 molecule to initiate the activation process. The relative amount of each of the molecules can alter the amount of pro-MMP-2 that is activated.

Table 1

Primer Sequences

Gene		Primer Sequence (5'–3')
<i>MTI-MMP</i>	Sense	GGCCTACCTGCTTCCATCAACAC
	Antisense	ACCCTCTGGGAGACTCAGGGATT
<i>MMP-2</i>	Sense	GACGATATCAAGGGCATTCAAGA
	Antisense	TTCTTGCTCCAGTTAAAAGCAGC
<i>MMP-3</i>	Sense	CAGGACACCTGAACCTGAATTTT
	Antisense	ACAGCATCAACCTTTGGTTCAAC
<i>TIMP-1</i>	Sense	CCTGGTCATCAGAGCCAAGTTCA
	Antisense	TCCTCACAGCCAGCAGCGTAG
<i>TIMP-2</i>	Sense	TCAAAGGACCTGACAAGGACATAGAG
	Antisense	CTTCTCTTGATGCAGGCGAAGA
<i>TIMP-3</i>	Sense	CCAAGGTGGTGGGAAAAGAAGC
	Antisense	AGGTCACAAAGCAAGGCAGGTA
<i>18s</i>	Sense	GTTTTCGGAAGTGAAGGCCATGA
	Antisense	TGCCCTTCCGTCAATTCCTTTA