

## *Invited Review*

# **Repair in avascular tissues: fibrosis in the transparent structures of the eye and thrombospondin 1**

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**Summary.** Wound repair is a process which is normally dependent on the vasculature of the damaged tissue. However, the transparent structures of the eye (e.g. central cornea, lens, vitreous) are avascular and yet are still subject to repair and fibrosis. Moreover, the resulting ophthalmic scars often remain avascular. Since this type of ocular scarring may result in blindness, it is the subject of intense research. An aspect of avascular ophthalmic fibrosis which has attracted attention is the question concerning early wound healing components that are usually derived from blood constituents. One such molecule is the glycoprotein thrombospondin 1. Thrombospondin 1 is thought to be a key regulator of cell behaviour in early wound repair and appears to be derived totally from platelet  $\alpha$ -granules during repair of incisional skin wounds. It has been shown that the ocular cells involved in avascular repair processes, and which are thus responsible for healing in the absence of platelet-derived thrombospondin 1, are capable of synthesizing the protein themselves. It is suggested that cells involved in ophthalmic repair processes produce thrombospondin 1 in the absence of the platelet-derived molecule. Local synthesis of thrombospondin 1 may represent a therapeutic target in the management of ophthalmic fibrosis.

**Key words:** Cornea, Lens, Retina, Wound healing, Thrombospondin

### **1. Introduction**

Except for the most minor injuries in which regeneration of parenchymal cells alone is sufficient to replace damaged tissue, healing of wounds requires repair with fibrous tissue. In general all stages of fibrous tissue formation, from the initial fibrinous blood clot to the granulation tissue which produces the final scar, are dependent on the local vasculature. Nevertheless,

wounds in the avascular tissues of the eye such as the central cornea typically remain free of new blood vessels and yet still heal by fibrosis. Although avascular ocular fibrosis provides an opportunity to examine aspects of wound healing in isolation from vascular elements, the process raises questions about the biology of early fibrosis in the absence of blood and its constituents. For example, since one blood component usually pivotal to early repair is the platelet, the question arises as to whether platelet-derived molecules are involved in repair in avascular wounds. Such inquiries are the more germane when it is considered that avascular ocular fibrosis can have a disastrous effect on vision. To construct a logical therapeutic approach to these complications, there is a need for a clear understanding of early ocular repair. This article will examine some features of avascular ocular repair, with particular reference to early corneal scarring and the platelet glycoprotein thrombospondin 1 (TSP1).

### **2. Ophthalmic wound repair - impetus and vehicle for research**

Injuries to the eye are common. In the past, severely damaged eyes were usually rapidly removed (enucleated) to prevent the development of sympathetic ophthalmitis (a condition in which the fellow eye becomes damaged by a destructive immune disease of unclear pathogenesis) (Foster, 1994). With the advent, however, of microsurgery and steroids, severely traumatised eyes are now conserved whenever possible. Nevertheless, despite surgical and medical intervention, badly damaged eyes may eventually be lost because of wound healing problems.

The complications of ocular wound healing, like those of wounds elsewhere in the body, include inadequate, excessive and inappropriate repair. Inadequate repair may lead to failure of the eye to regain its integrity as a closed organ, which in turn may result in intraocular infection (endophthalmitis) or ingrowth of cells normally present only at the ocular surface (such as epithelial ingrowth - Fig. 1). Indeed, epithelial ingrowth itself may be regarded as an inappropriate repair response. However, probably the major complication of

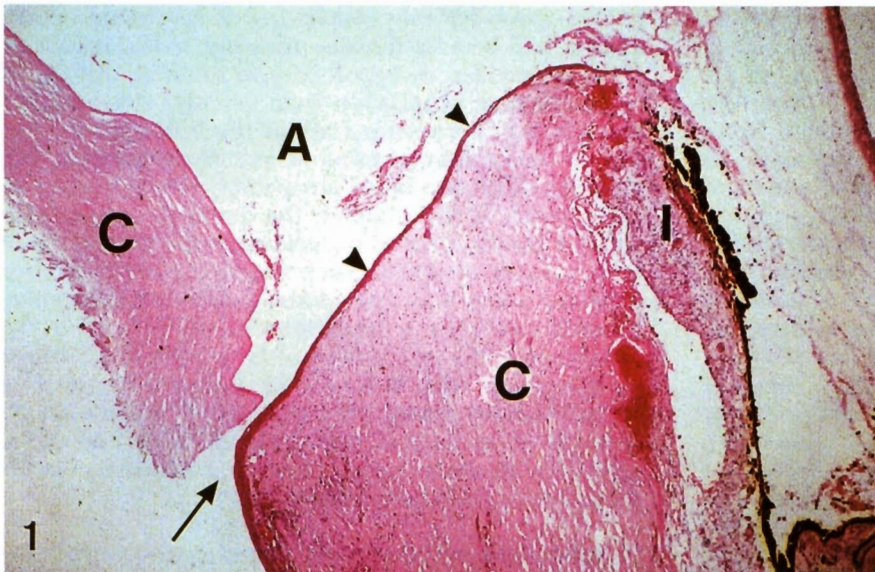
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ocular repair is inappropriate or excessive fibrosis (for a review see Grierson et al., 1997).

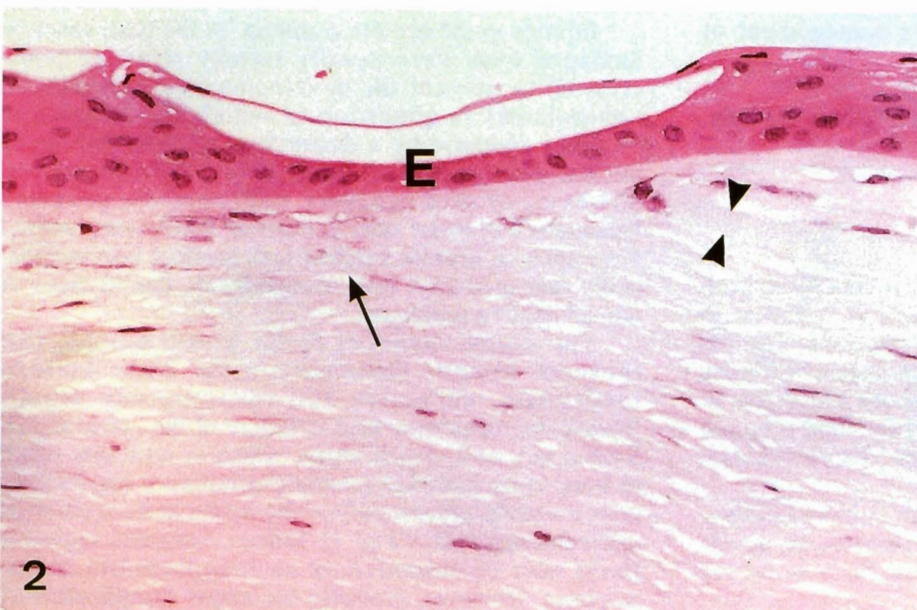
Fibrosis causes problems either by tractional distortion of ophthalmic structures or by causing opacification of the ocular media (Grierson et al., 1997). Thus fibrosis in the cornea may cause traction-mediated deformity of the light-refracting surfaces (severe astigmatism) or failure of refractive surgery (Luttrull et al., 1985), while opaque central corneal fibrosis impedes light entry (Fig. 2). Indeed, post-trachomatous corneal scarring of childhood is the commonest cause of blindness from infection worldwide, and corneal scars are one of the commonest causes of preventable blindness in England and Wales (Evans et al., 1996).

Opaque fibrosis involving the lens components or vitreous also restricts vision, while contraction of fibrous tissue on the retinal surface may cause retinal distortion and/or detachment (Grierson et al., 1997). Tractional detachment of the ciliary body reduces the production of aqueous humour, lowers intraocular pressure and ultimately may cause ocular shrinkage and disorganisation (phthisis bulbi).

The complications of ocular wound healing are difficult to treat and the effort to improve the management of fibrosis in the eye has stimulated analysis of the basic mechanisms of ophthalmic repair, such as the biology of early fibrosis in the absence of blood and its constituents. Paradoxically, the effects of



**Fig. 1.** Haematoxylin and eosin stained section through part of the anterior segment of a human eye. The cornea (C), anterior chamber (A) and iris (I) are indicated for orientation purposes. There is a full-thickness wound in the cornea (arrow) and the iris has become adherent to the back of the cornea. The wound has failed to heal and surface epithelium has grown into the eye to form an epithelial ingrowth (arrow heads), x 40



**Fig. 2.** Section through part of the anterior surface of a human cornea. The epithelium (E) is irregular in thickness. The anterior acellular condensation of the stroma known as Bowman's layer has mostly been destroyed, leaving only a small piece of the layer (arrowheads). Avascular fibrous tissue has filled the defect in Bowman's layer and replaced the normal architecture of the anterior stroma with an opaque scar (arrow). Some of the avascular fibrous tissue extends between the epithelium and the anterior surface of Bowman's layer. Haematoxylin and eosin, x 400

blood components on repair and angiogenesis are sometimes investigated in the avascular tissue of the cornea. This "corneal *in vivo* angiogenesis model" depends on vascular ingrowth into the otherwise avascular cornea following the implantation of test substances. The substances are placed in pellets which are surgically implanted into a corneal wound and the effects of the agents on new vessel formation are subsequently monitored.

One molecule which has been studied in the corneal "in vivo model" is the platelet glycoprotein thrombospondin 1 (TSP1) (reviewed by Tuszynski and Nicosia, 1996). TSP1 is a large (420kD) homotrimeric molecule and a member of a family of at least five proteins (TSP1-4 and TSP5 or cartilage oligomeric protein) (see recent review by Adams, 1997). TSP1 is involved in a variety of biological processes, including wound healing (Raugi et al., 1987; Reed et al., 1993; Gailit and Clark, 1994; DiPietro et al., 1996). Although the precise role of TSP1 in wound healing is unclear, there is evidence that the protein plays a key role in permitting partial separation of cells from their surroundings, a function which would in turn allow the cellular proliferation and migration necessary for repair (reviewed by Sage and Bornstein, 1991; Gailit and Clark, 1994; Bornstein, 1995). Although two other proteins (tenascin and osteonectin: osteonectin sometimes is called SPARC - Secreted Protein Acidic and Rich in Cysteine) also have been implicated in promoting partial cell detachment (Sage and Bornstein, 1991), TSP1 appears earlier than SPARC or tenascin in skin wounds (Reed et al., 1993; Gailit and Clark, 1994). Moreover, knocking out TSP1 severely disrupts cutaneous wound healing in mice (Polverini et al., 1995). Thus TSP1 seems to be essential for normal repair.

### 3. Thrombospondin 1 and repair in avascular ocular tissues

TSP1 is a major component of platelet  $\alpha$ -granules (Baenziger et al., 1971; Lawler, 1986; Bornstein, 1992) and there is evidence that in cutaneous repair the glycoprotein is derived largely from platelets but not fibroblasts (Raugi et al., 1987; Reed et al., 1993; Gailit and Clark, 1994; DiPietro et al., 1996). However, fibroblastic cells rather than platelets predominate at the site of avascular ophthalmic repair and thus platelet-derived TSP1 would not be expected in such wounds. A number of possibilities arise with respect to TSP1 in these circumstances. For example, an alternative source of TSP1 may be available such as a local reservoir in the tissue. Another possibility is that TSP1 may be produced locally following injury - for example by infiltrating macrophages (which synthesise TSP1 after excisional but not incisional skin wounds: Reed et al., 1993; DiPietro et al., 1996) or resident cells adjacent to the damaged area (a wide range of cells are capable of making thrombospondin *in vitro*) (Raugi et al., 1982). On the other hand TSP1 may not be required in, and

hence may be absent from, non-vascularised repair.

To address some of these issues, we have investigated the relationship between TSP1 and ocular avascular repair. Our studies chiefly have concentrated on the central cornea, although we have also extended these studies to TSP1 in the lens and to the interface between the vitreous and retina (where a form of avascular "fibrosis" may occur - see below).

### 4. The cornea and thrombospondin 1

Other than in minor traumatic loss of the corneal epithelium (corneal abrasion) where regeneration of the epithelium alone is sufficient to close the defect, corneal wounds involve the stroma and thus require repair with fibrous tissue. Key players in this repair are the corneal stromal cells or "keratocytes". Keratocytes are sometimes also known as corneal fibroblasts, but they differ from cutaneous fibroblasts in that they are derived from neural crest components (reviewed by McCartney, 1994; Bron et al., 1997) and normally are arranged in a syncytium of stationary cells.

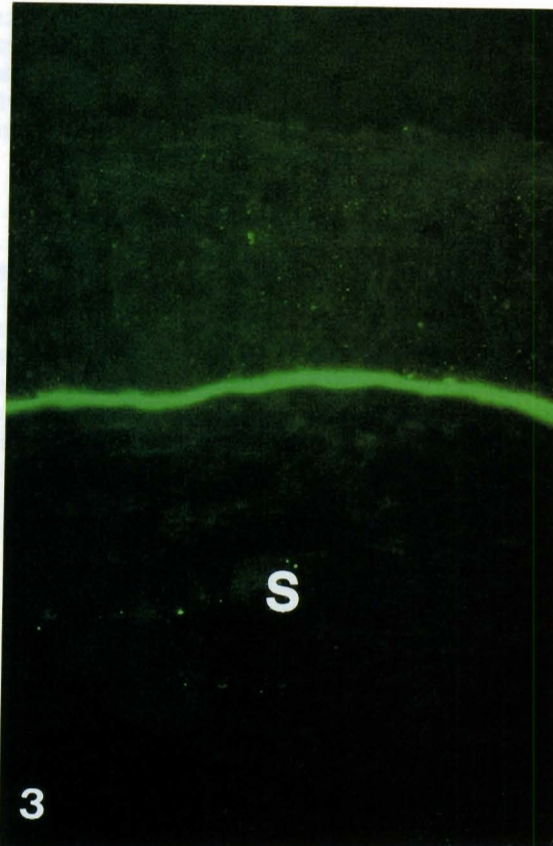
The initial stages of corneal stromal repair are characterised by degeneration of keratocytes in a zone up to 300  $\mu$ m around the wound (Matsuda and Smelser, 1973). Some of this stromal cell loss probably is a consequence of apoptosis secondary to damage of the overlying epithelium (see review by Wilson and Kim, 1998). It has long been established that surviving keratocytes adjacent to the wound lose their processes and syncytial arrangement within one hour of injury (Duke-Elder and Leigh, 1965). These keratocytes become isolated, motile cells.

Given that TSP1 is thought to be a major facilitator of cell migration, the change in keratocyte phenotype following injury might be anticipated to be TSP1-dependent. As there is no vascular source of TSP1 in the corneal stroma, we looked for a local reservoir of TSP1 in the stroma and the rest of the cornea.

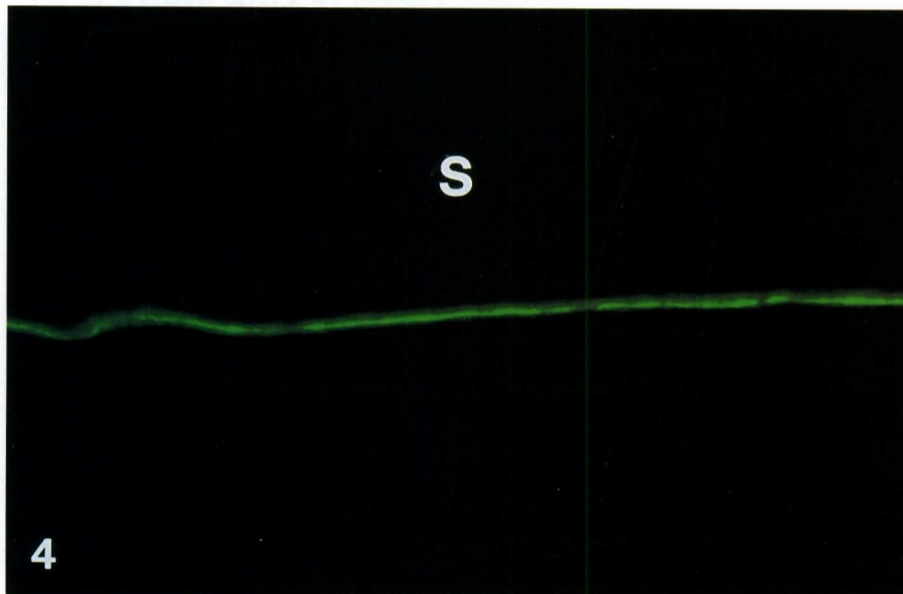
#### 4.1 TSP1 in normal cornea

In studies of TSP1 in the eyes of several species (Table 1), we employed antibodies raised against human platelet thrombospondin. Platelet thrombospondin probably consists solely of TSP1 (Bornstein, 1992). TSP1 is, however, similar to thrombospondin 2 so that polyclonal antibodies may not be able to distinguish between the two molecules (Bornstein, 1992). We therefore used only monoclonal antibodies to platelet thrombospondin (TSP1) in our investigations. Such antibodies may react with bovine as well as human TSP1 (Dreyfus and Lahav, 1988).

We were unable to detect TSP1 in immunoblots of corneal stromal extracts, even when the extracts were prepared with sodium dodecylsulphate (unpublished data). Because extraction of matrix-bound thrombospondins can be difficult (Mosher, 1990), corneal TSP1 was further studied using light and electron microscopic



**Fig. 3.** Immunofluorescence labelling of TSP1 in corneal epithelial basement membrane in a frozen section of bovine cornea. The epithelial and stromal tissues (S), including anterior keratocytes, are not immunoreactive for TSP1. x 600



**Fig. 4.** Immunofluorescence staining of TSP1 in frozen sections of human cornea. The endothelium contains TSP1 (seen as bright linear fluorescence) but the stromal tissue (S), including posterior keratocytes, does not stain for the glycoprotein. x 500

immunohistochemistry (Hiscott et al., 1997).

Immunohistochemical evaluation of TSP1 in the normal cornea failed to detect the glycoprotein in the stroma or keratocytes. However, TSP1 was found in the epithelial basement membrane and in the endothelium (Figs. 3, 4; Table 1). The epithelial basement membrane labelling was continuous with inconstant TSP1 immunoreactivity in the basement membrane at the limbus, while the endothelial staining was continuous with TSP1 immunostaining in the trabecular meshwork. Interestingly, some species variation was found in these results. That is, although human and bovine epithelial basement membrane and endothelium were immunoreactive for TSP1, no staining was observed in rabbit or porcine cornea (Table 1). The absence of TSP1 staining in the cornea of the latter two species may indicate variations in TSP1 between species with regard to antigenic sites

**Table 1.** The distribution of thrombospondin 1 immunoreactivity in the cornea.

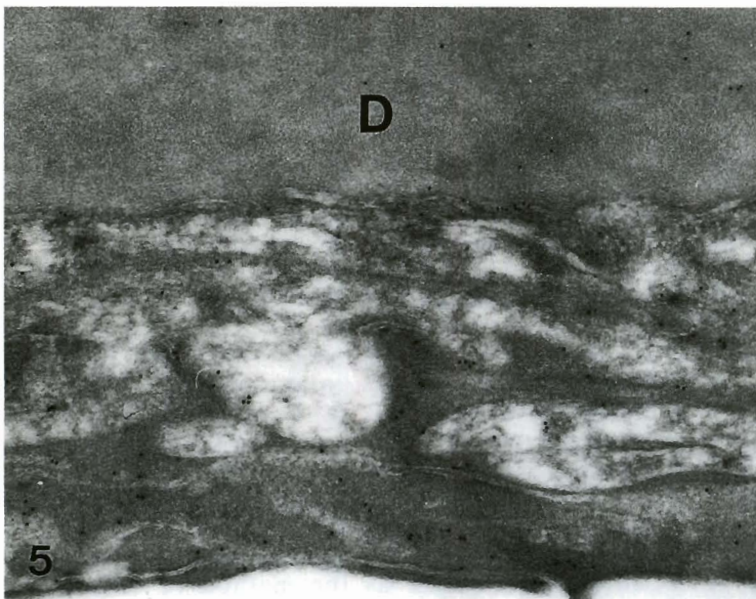
	HUMAN	BOVINE	LAPINE	PORCINE
Epithelium	-	-	-	-
Epithelial basement membrane	+*	+*	-	-
Stromal cells (keratocytes)	-	-	-	-
Stroma (matrix)	-	-	-	-
Descemet's membrane	+ <sup>a,b</sup>	+ <sup>a,b</sup>	-	-
Endothelium	+	+	-	-

+: immunoreactivity for TSP1; -: background labeling only; \*: inconsistent TSP1 labelling in tissue immediately adjacent to epithelial basement membrane, this staining was much less intense than seen in the membrane; <sup>a</sup>: adjacent to endothelium, i.e. posterior Descemet's membrane only; <sup>b</sup>: inconsistent TSP1 labelling of posterior stroma, Descemet's interface at level much less intense than that seen in the endothelium or posterior Descemet's membrane.

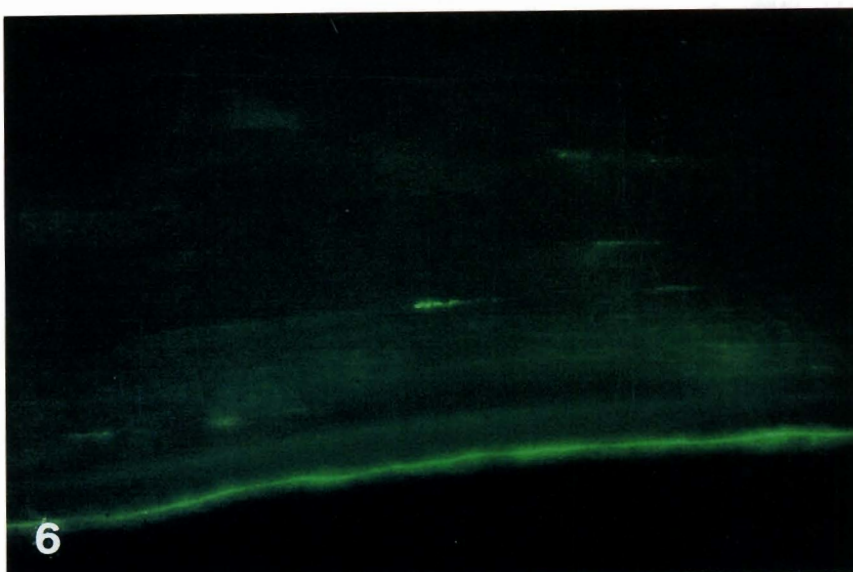
recognised by antibodies, since not all antibodies (or even antisera) raised to human or bovine TSP1 cross-react with TSP1 of other species (Raugi et al., 1982; Jaffe et al., 1983). On the other hand, a thrombospondin 2 (TSP2) clone has been isolated in a study employing a rabbit corneal endothelial cDNA library (Hotta et al., 1997).

The finding of TSP1 in human and bovine corneal epithelial basement membrane and endothelium is consistent with observations of TSP1 antisera labelling of a variety of basement membranes (Wight et al., 1985), including that of the epidermis (the corneal epithelium derives from surface ectoderm), and corneal endothelium of the rat and cow (Munjal et al., 1990; Gordon,

1994) respectively. However, the results of our electron microscopic studies differ from those of the latter study. Thus our investigations (Hiscott et al., 1997), undertaken to clarify the subcellular distribution of TSP1 in the posterior cornea, revealed TSP1 not only within the corneal endothelium but also in posterior Descemet's membrane (the "youngest" part of the membrane since the membrane accrues throughout life: Gipson, 1994) (Fig. 5). However, the rest of Descemet's membrane did not label for TSP1 (Hiscott et al., 1997). The studies of Munjal and colleagues (which employed thrombospondin antisera) demonstrated labelling primarily at the posterior surface of the endothelium and not within Descemet's membrane (Munjal et al., 1990). These



**Fig. 5.** Transmission electron micrograph of interface between posterior Descemet's membrane (D) and bovine corneal endothelium (below). The tissue was stained by a previously described immunogold method (Schlötzer-Schrehardt and Dörfler, 1993) for TSP1. The endothelium and posterior Descemet's membrane both label for TSP1. x 35,000



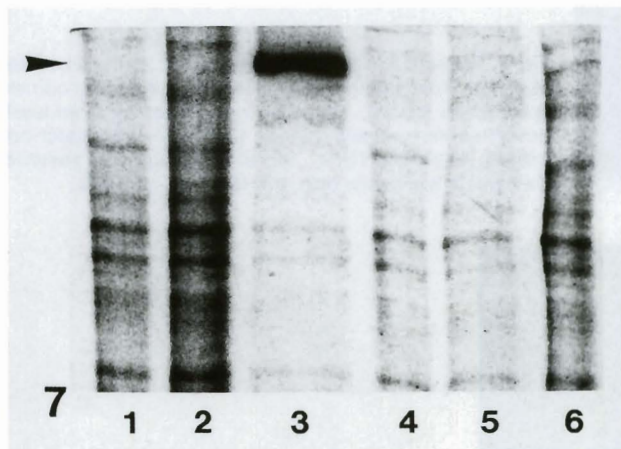
**Fig. 6.** Immunofluorescence staining for TSP1 in frozen sections of posterior human cornea from a patient with the pseudoexfoliation syndrome. The endothelium is seen as bright linear fluorescence. Note that the keratocytes in the stroma (upper part of figure) express TSP1. x 500

differences may reflect methodological variations between the investigations of Munjal and coworkers, who employed a pre-embedding immunoperoxidase technique, and our post-embedding immunogold study.

#### 4.2 TSP1 in damaged cornea

Munjal and colleagues provided evidence that corneal endothelial cells have the ability to produce TSP1 and that this synthesis is greatly increased following endothelial injury (Munjal et al., 1990). Thus endothelial-derived TSP1 represents a potential source of the glycoprotein following corneal damage. The presence of TSP1 in the basement membrane of the corneal epithelium suggests that the epithelium could also supply TSP1 following injury. Yet another potential TSP1 source is the tear film, although it has been reported that tear TSP1 levels are very low (Crombie et al., 1998). Additionally, in corneal injury (or inflammation) TSP1 could be synthesized by infiltrating inflammatory cells.

Tear-, epithelial- or endothelial-derived TSP1 might reach the damaged stroma if an injury breaching either the anterior or posterior corneal surface tracks into the corneal stroma. However, damage may occur to the stroma in the absence of such penetrating injuries. The common though little known degenerative ocular disease known as pseudoexfoliation syndrome is associated with a keratopathy (Naumann et al., 1995). We have observed



**Fig. 7.** Autoradiograph of immunoprecipitated TSP1 from cultured bovine keratocytes, separated in SDS-PAGE under reducing conditions (after the method of Sorokin et al., 1994). Preconfluent (lanes 1 to 3) or confluent (lanes 4 to 6) cultures of the cells were labelled with  $^{35}\text{S}$  methionine plus  $^{35}\text{S}$  cysteine. Immunoprecipitation was subsequently performed on cell lysates using anti-TSP1 monoclonal antibody (lanes 3 and 6). As controls, immunoprecipitation was also undertaken without antibody (lanes 1 and 4) or with an inappropriate monoclonal antibody (lanes 2 and 5). The precipitates were separated in SDS-PAGE under reducing conditions prior to autoradiographic detection. The arrow marks the 185kD position (the size of reduced TSP1). The keratocytes in preconfluent culture have synthesised TSP1 (lane 3), but not in confluent culture (lane 6). The control precipitates demonstrate that several bands are non-specifically bound to Sepharose protein-A.

TSP1 staining of keratocytes within the corneal stroma in this syndrome (Hiscott et al., 1996a) (Fig. 6). As in other normal corneal tissue (see above), keratocyte staining for TSP1 was absent from the keratocytes of age-matched controls (Hiscott et al., 1996a). Since the surfaces of the cornea are usually intact in pseudoexfoliation syndrome, tear- and/or epithelial-derived TSP1 would have to pass through the dense acellular zone of the anterior stroma known as Bowman's layer while endothelial-derived TSP1 would have to permeate Descemet's membrane. Moreover, as thrombospondins bind tightly to matrix (Mosher, 1990), it seems unlikely that keratocyte-associated TSP1 is derived from tears, epithelium and/or endothelium. Furthermore, pseudoexfoliation keratopathy primarily involves the endothelium (Naumann et al., 1995). Thus keratocyte-associated TSP1 is unlikely to originate from inflammatory cell infiltration of the stroma in the syndrome (see also sections 4.3 and 4.4 which concern studies of isolated keratocytes). In addition, there is cogent evidence that a cell must be actively synthesising TSP1 for it to bind the glycoprotein (summarised by Lahav, 1993). Taken together, these observations suggest that keratocytes themselves are able to produce TSP1 and do so in pseudoexfoliation syndrome.

#### 4.3 Keratocytes can make TSP1 in vitro

In view of the importance of TSP1 in repair processes and of its potential role in corneal stromal responses to injury, we further investigated whether keratocytes are capable of producing TSP1. This investigation used cultured keratocytes.

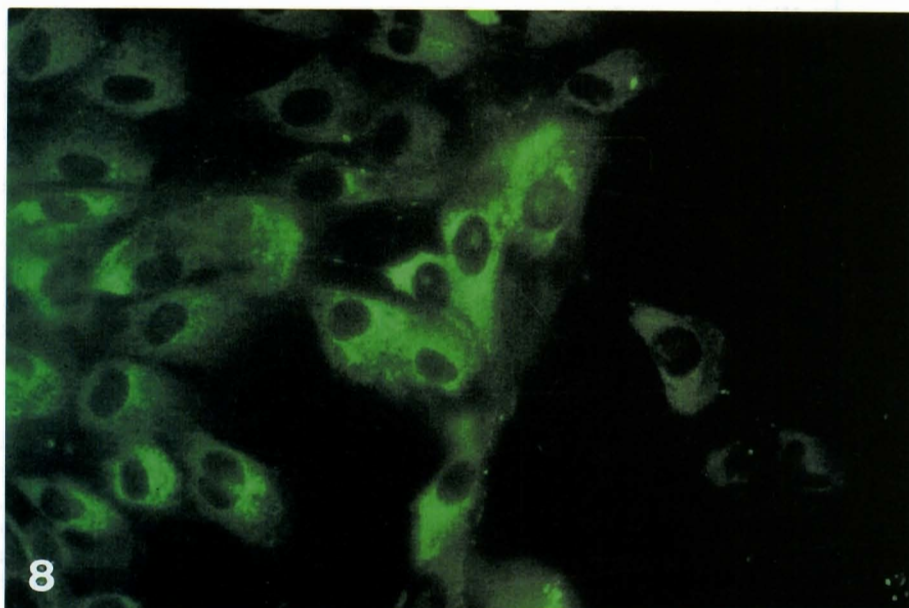
Keratocyte cultures are often obtained from discs of cornea which have been scraped to remove epithelial and endothelial cells, then minced and treated with collagenase (such as the method of Woost and colleagues, 1985). We modified this method by obtaining stromal explants from unopened globes (Hiscott et al., 1996b). The corneal epithelium was removed with a scalpel blade and alcohol, the central stroma was trephined (6mm diameter) to three quarters stromal depth and a lamellar stromal disc excised with right-angle surgical scissors. Eyes in which the cornea was perforated were discarded (to avoid culture contamination by endothelial cells). The stromal lamellar discs were dissected into 1mm<sup>2</sup> fragments without collagenase. From these pieces, keratocytes were easily propagated in standard culture flasks and medium. This method avoided the chance of endothelial or limbal cell contamination, and negative immunostaining for cytokeratins and the kite- and spindle-shaped morphology of cells in preconfluent culture confirmed there was no epithelial contamination (Hiscott et al., 1996b).

The cultured keratocytes were examined for evidence of TSP1 synthesis by two methods (Hiscott et al., 1996b). Firstly, the cells were studied by immunoprecipitation following metabolic labelling with

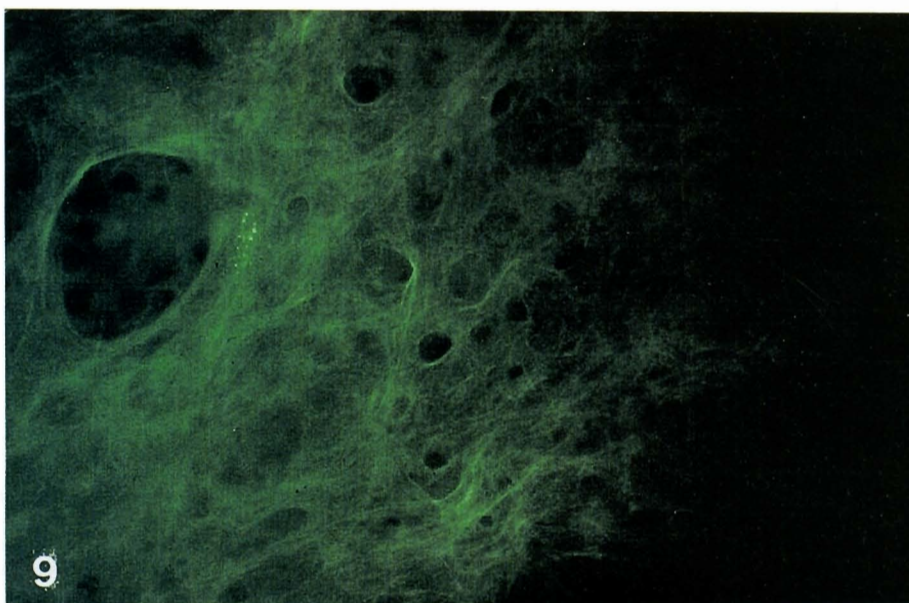
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methionine and cysteine. Secondly, in view of the evidence that cells which bind TSP1 also are producing the protein (see above), keratocyte monolayers were investigated with immunocytochemical methods for TSP1 expression. These two approaches yielded essentially similar findings. Keratocytes in preconfluent culture synthesise TSP1 (Figs. 7, 8). However, postconfluent cells did not appear to produce the protein, although TSP1 staining could still be detected in the extracellular matrix around postconfluent cells (Figs. 7, 9). Our initial studies employed bovine keratocytes, but we have subsequently achieved similar results with human cells.

Consistent with our findings concerning TSP1 in the rabbit cornea (see above), we could not demonstrate TSP1 production by lapine keratocytes either by immunoprecipitation or immunocytochemistry with monoclonal antibodies raised against human TSP1. Nevertheless, despite this negative staining, in postconfluent rabbit keratocyte cultures the extracellular matrix did stain for the protein (Fig. 10). There is evidence that TSP1 is incorporated into the matrix deposited by some cultured cells (Jaffe et al., 1983; Dreyfus and Lahav, 1988) and that the molecule itself is present in fetal calf serum (Raugi et al., 1982). Since fetal calf serum was used to culture our rabbit kerato-



**Fig. 8.** Bovine keratocytes in preconfluent culture labelled by the immunofluorescence method for TSP1. Most of the cells exhibit intense cytoplasmic staining. x 600



**Fig. 9.** TSP1 immunofluorescence staining of a postconfluent bovine keratocyte culture. The cells are not immunoreactive for TSP1 but there is focal staining of the extracellular matrix. x 500

cytes, the TSP1 immunoreactivity of the lapine keratocyte extracellular matrix probably reflects fetal calf serum-derived TSP1 bound to lapine keratocyte matrix.

The lack of TSP1 synthesis by human or bovine keratocytes in confluent culture and in normal cornea suggests that keratocytes in a syncytial arrangement do not produce the protein. Conversely, TSP1 production by keratocytes in damaged corneal stroma and pre-confluent cultures intimates that isolated, motile cells do produce TSP1. Moreover, there is evidence that secretion of TSP1 by other cell types in culture is cell density-dependent (Mumby et al., 1984; Dreyfus and Lahav, 1988). Cell density-dependent TSP1 secretion by keratocytes would be in keeping with the concept that there is a relationship between the "wound healing" keratocyte phenotype and TSP1 production. We have further investigated this apparent association using two *in vitro* models of corneal stromal wounds.

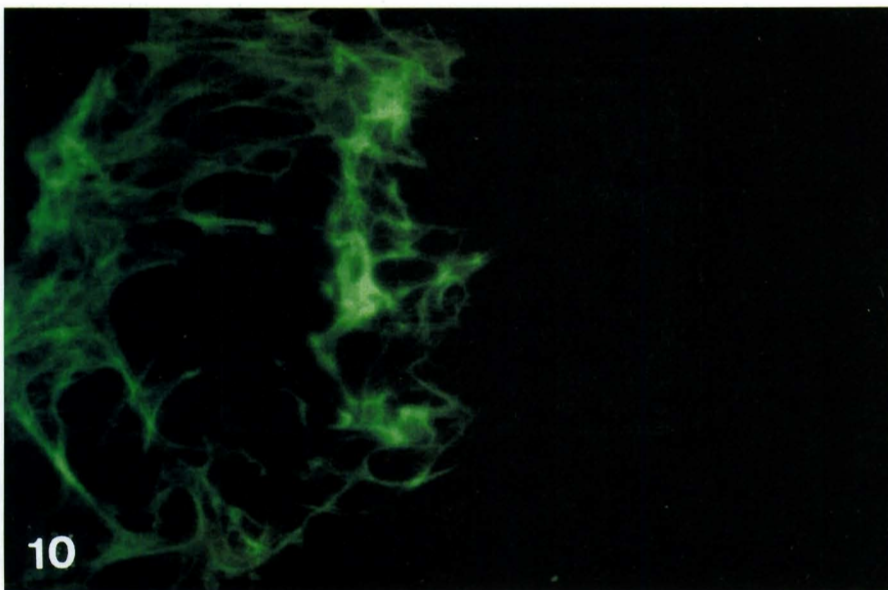
#### 4.4 TSP1 and *in vitro* models of wounds of the corneal stroma

Cell-populated hydrated collagen matrices have been used as a model in which to investigate aspects of wound repair (including ocular repair) such as tissue contraction and cell migration (Bell et al., 1979; see also review by Ehrlich, 1988). With respect to the cornea, this three-dimensional system has been employed by several groups to examine human or bovine keratocyte response to stromal injury (Assouline et al., 1992; Hiscott et al., 1996b). We have used keratocyte-populated collagen matrices to further investigate the relationship between keratocyte expression of TSP1 and the behaviour of the cells. In collagen matrices, cells are migratory but do not proliferate and therefore the method permits evaluation of motile rather than replicating cells. Motile keratocytes

in the matrices are immunoreactive for TSP1 (Hiscott et al., 1996b). Moreover, electron microscopic immunogold studies revealed TSP1 at sites of apparent contact between keratocyte and collagen fibres, in cell surface invaginations and intracellularly (Hiscott et al., 1996b). This subcellular location of TSP1 is similar to that proposed for cells producing the protein during interactions with a substrate and is consistent with a function for TSP1 in regulating such keratocyte activities as migration (Vischer et al., 1988; Völker et al., 1991; Tooney et al., 1993).

An alternative model for investigating stromal repair is that of the organ-cultured cornea. In a method described by Collin and colleagues (Collin et al., 1995), corneal wounds are created in excised (enucleated) whole eyes and then the cornea is dissected immediately from the globe and placed in air/liquid organ culture for up to 12 days. In initial experiments to determine whether and when keratocytes begin to express TSP1 after stromal wounding, we modified this organ culture method by leaving the cornea *in situ* after non-perforating (two-thirds stromal depth) linear mechanical wounds (to mimic the *in vivo* state more closely). The specimens were maintained for only a short time in culture (up to 24 hours), using a serum free medium to avoid contamination from serum TSP1 (see above). Preliminary results with bovine cornea indicate that TSP1 immunoreactivity first appears in keratocytes adjacent to the injury about 4 hours after wounding (Fig. 11). Investigations using this model are continuing.

The evidence from studies of normal and damaged corneal stroma and from *in vitro* investigations cogently suggests that normal keratocytes do not express TSP1, but that following a stromal injury the cells begin to produce the protein. Since keratocytes are involved in avascular stromal repair, our findings suggest that local



**Fig. 10.** The extracellular matrix of rabbit keratocytes labels for TSP1 in an immunofluorescence stained postconfluent culture. The cells, however, do not stain for the glycoprotein. x 500



production of TSP1 represents an alternative source to platelet-derived TSP1 during wound healing in non-vascularised stroma.

### 5. Thrombospondin 1 and other avascular reparative phenomena

Two other ocular sites which are involved in avascular fibrosis are the lens and the vitreoretinal interface. Processes at both these sites are thought to involve mesenchymal transdifferentiation of epithelial cells.

#### 5.1 TSP1 and fibrosis of the lens capsule

In the lens, epithelial cells may become fibroblastic following injury. This fibroblastic transformation is most often seen after cataract extraction when newly produced fibrous tissue causes opacification of the residual (posterior) lens capsule with visual reduction. Indeed, capsular fibrosis is the commonest cause of reduced vision after such surgery. Although we have not looked for TSP1 in these lenticular scars, we have shown that normal adult lens epithelial cells are immunoreactive for TSP1 and that cultured lens epithelial cells synthesise the protein (Fig. 12) (Hiscott et al., 1996c). These observations are consistent with previous indications that embryonic lens epithelium, like other epithelia, contains TSP1 (O'Shea and Dixit, 1988; Corless et al., 1992). There is, however, a difference between lens and many other epithelia with regard to TSP1. Whereas thrombospondin immunoreactivity (as detected with an antiserum to human platelet thrombospondin) typically is restricted to epithelial basement membranes in adulthood (Wight et al., 1985), in the lens TSP1 remains within the epithelium and does not appear to accumulate

in the lens epithelial basement membrane (Hiscott et al., 1996c). In this respect, the lens capsule is similar to the renal glomerulus and Bowman's capsule which also lack consistent, immunohistochemically detectable TSP1 (Wight et al., 1985).

Whatever the distribution of TSP1 in the lens, there is evidence that the molecule is involved in cell-cell interactions (see Lahav, 1993 for a review) and thus it is possible that TSP1 regulates associations between normal adult lens epithelial cells. Since cultured lens epithelia continue to produce TSP1, it seems likely that the glycoprotein also plays a role in capsular fibrosis.

#### 5.2 TSP1 and fibrosis at the vitreoretinal interface

Although studies in our laboratory have not detected TSP1 in normal adult retina (Hiscott et al., 1999), the glycoprotein is present in the scar-like tissues which form on the surfaces of the sensory retina in the blinding condition known as proliferative vitreoretinopathy (Fig. 13) (Esser et al., 1991; Hiscott et al., 1992, 1999). This disorder is thought to be an anomalous reparative response which arises as a complication of retinal detachment (and it is the commonest cause of failure of detachment surgery) (Hiscott and Grierson, 1994). It is characterised by the formation of contractile fibrocellular "membranes" containing metaplastic retinal pigment epithelium, glia and other cells. Vascular elements generally are absent from these membranes (Hiscott et al., 1985; Hiscott and Grierson, 1994) and macrophages also are not a consistent constituent of this type of fibrosis (Charteris et al., 1992, 1993). Thus neither platelet nor macrophage-derived TSP1 are likely to contribute to the pathology.

Available data indicate that retinal pigment epithelial cells which have dedifferentiated to a fibroblastic pheno-



**Fig. 11.** Differential interference contrast micrograph of a section through bovine cornea. The cornea received a linear mechanical wound (W) in organ culture. After 4 hours, the cornea was fixed and then embedded in glycol methacrylate resin (Kanawati et al., 1996). Sections were stained by the immunoperoxidase method (no counterstain) to show TSP1 brown. Keratocytes near the wound are seen to stain for TSP1 (arrowheads). Some focal staining in the epithelium (E) is also apparent. x 500

type contribute to the TSP1 in this type of retinal fibrosis (Hiscott et al., 1998, 1999), though glial cells are capable of TSP1 synthesis (Asch et al., 1986; Scott-Drew and French-Constant, 1997) and therefore may also be involved in production of the glycoprotein in the membranes. Nevertheless, glial cells are usually not such a prominent component of these membranes as retinal pigment epithelial cells (Hiscott et al., 1999). Furthermore, epithelial cells have been implicated in TSP1 synthesis in aberrant fibrosis elsewhere in the body such as pulmonary fibrosis (Kuhn and Mason, 1995).

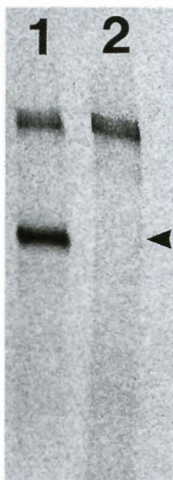
Unlike cutaneous wounds, the fibrosis in proliferative vitreoretinopathy evolves over many months with evidence of persistent cell recruitment and proliferation (Hiscott et al., 1985). Although TSP1 is generally considered as an early wound repair molecule

in skin (Gailit and Clark, 1994), the glycoprotein can be found even in longstanding retinal scars (Fig. 13). This observation is consistent with the perception that TSP1 plays a role in the key cellular activities of migration and proliferation during wound repair.

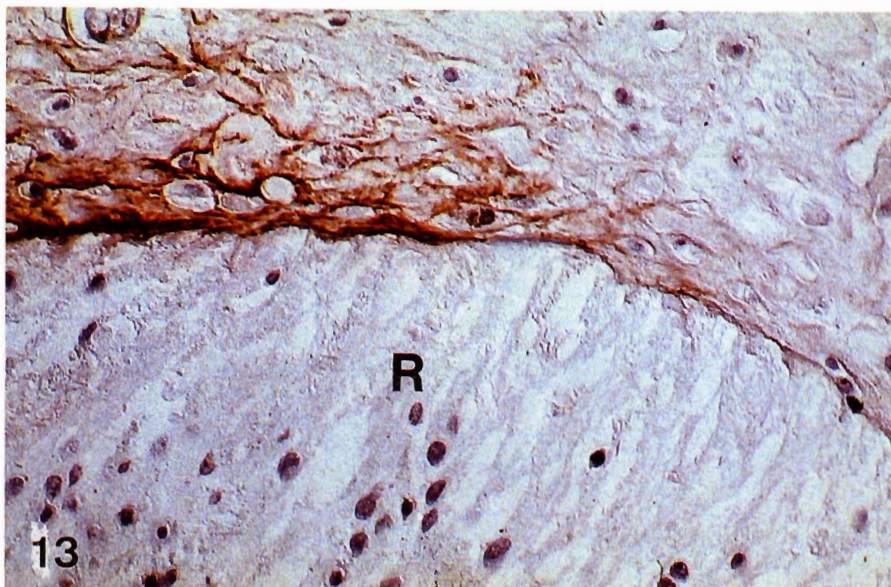
## 6. Conclusions and future directions

Studies of reparative phenomena in avascular ocular tissues indicate that local cells are capable of synthesising an early wound repair molecule, TSP1, which in cutaneous wounds is probably chiefly derived from the vasculature. One possible explanation for the difference between cutaneous and ocular repair with regard to local TSP1 production is that in cutaneous wounds platelet-derived TSP1 might suppress local production whereas in avascular ophthalmic repair platelet-derived TSP1 is not available to restrict such synthesis. Indeed, several of the cell types involved in cutaneous wound repair are capable of TSP1 synthesis *in vitro* (Raugi et al., 1982; DiPietro et al., 1996).

Local synthesis of molecules not normally found in tissues may present a therapeutic target in the control of repair and the problems which fibrosis can cause. In the eye this may apply particularly to TSP1 production in scarring of the corneal stroma and vitreoretinal interface. An experimental approach to excisional skin wounds has shown that modulation of macrophage-derived TSP1 can modify healing (DiPietro et al., 1996). The eye is easily accessible and avascular ophthalmic fibrosis tends to occur in the enclosed cavities of the vitreous or conjunctival sacs. These characteristics suggest that local therapy, similar to that shown to be effective for excisional wounds, is a real possibility for the future management of complicated ophthalmic repair.



**Fig. 12.** Immunoprecipitation of TSP1 from the conditioned media of bovine lens epithelial cells. Cultures of cells were labelled with  $^{35}\text{S}$  methionine plus  $^{35}\text{S}$  cysteine and immunoprecipitation was performed using anti-TSP1 monoclonal antibody (lane 1). A similar precipitation was undertaken without antibody as a control (lane 2). The precipitates were separated in SDS-PAGE (under reducing conditions) prior to autoradiographic detection. The arrowhead marks the 185kD band. Lens epithelial cells have synthesised TSP1 and released the protein into their media (lane 1). The precipitate performed without antibody demonstrates that a band at 250kD represents non-specific binding of proteins to Sepharose protein-A (lane 2).



**Fig. 13.** Light micrograph from a  $2\ \mu\text{m}$  thick section through a glycol methacrylate resin embedded retina (R) stained by the immunoperoxidase method for TSP1 (haematoxylin counterstain). There is an avascular fibrous membrane on the vitreous surface of the retina. The epiretinal "scar" contains abundant TSP1 (labelled brown). The retina, however, shows no immunoreactivity for the glycoprotein.  $\times 300$

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