Role of Calcium-Dependent Protease(s) in Globulization of Isolated Rat Lens Cortical Fiber Cells

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PURPOSE. To investigate the role of calcium-activated proteases in calcium-dependent disintegrative globulization of isolated rat lens cortex fiber cells.

METHODS. Rat lens fiber cells were isolated and plated on coverslips at the bottom of a temperature-controlled chamber. The fiber cells were incubated with 10 μM protease substrate, (t-butoxycarbonyl-leu-met-7-amino-4-chloromethylcoumarin: BOC-Leu-Met-CMCA) and the proteolytic activity in the fiber cells was determined by observing the increase in fluorescence, using an excitation wavelength of 360 nm, and measuring emission at 410 nm. Free intracellular calcium was measured using the cell-permeable calcium indicator Fluo-3-AM, and the globulization time (Tg) was determined using image analysis.

RESULTS. Tg for fiber cells superfused with Ringer’s solution containing 2 × 10⁻³ M, 10⁻⁶ M, and 10⁻⁸ M [Ca⁺²], were: 24.7 ± 1.3, 53.0 ± 2.8, and more than 120 minutes, respectively. A significant increase in Tg (~ 95 minutes) was observed when the fibers were preincubated with acetoxymethyl ester of 1,2-bis (2-amino-phenoxy) ethane N, N, N, N-tetra-acetic acid (BAPTA-AM) to buffer changes in [Ca⁺²], or the protease substrate to competitively inhibit degradation of cellular proteins. In the presence of Ringer’s solution containing 2 × 10⁻³ M [Ca⁺²], and 0.5 mM of the cysteine protease inhibitor, leupeptin, Tg increased to 100 minutes, without affecting [Ca⁺²]. The proteolytic activity of fiber cells in Ringer’s solution containing 10⁻⁶ M and 2 × 10⁻³ M [Ca⁺²], increased by approximately 7- and 12-fold, respectively, compared with sucrose-EDTA solution or Ringer’s solution containing 10⁻⁸ M [Ca⁺²]. This increase in proteolytic activity was inhibited by leupeptin.

CONCLUSIONS. Elevation of calcium in the medium results in a proportionate increase in [Ca⁺²], and the proteolytic activity in isolated lens fiber cells. The increase in the proteolytic activity is accompanied by an increase in the rate of globulization of the fiber cells. Inhibition of the proteolytic activity by leupeptin increases Tg without affecting the gain in [Ca⁺²]. These results suggest that globulization of isolated fiber cells in physiological salt solutions is mediated by Ca⁺²-activated protease(s). (Invest Ophthalmol Vis Sci. 2001;42:194-199)

The lens consists of a single layer of epithelium, which differentiates into fiber cells. As the fiber cells differentiate, they elongate, join with fibers originating from the opposite equatorial region, and gradually lose their subcellular organelles. In the fully mature lens, the arrangement of elongated fibers in tight concentric layers minimizes light absorbance and scattering. Although such an arrangement of fibers is conducive for reducing light scattering, on differentiation, the fiber cells become metabolically less active. Furthermore, the restyling potential of the differentiated fiber cells is maintained by coupling to each other and to the epithelium through abundant gap junctions (for review see Reference 1 and references therein). We have recently developed procedures for isolating single fiber cells from rat lens cortex and have demonstrated that the isolated cells are viable as assessed by the exclusion of trypan blue. Moreover, the isolated fibers appear to maintain their gap junctions in the closed state, because they exclude the gap junction-specific dye Lucifer yellow.2–4 Furthermore, these cells remain viable in nonionic media, but when exposed to Ringer’s solution containing 2 × 10⁻³ M calcium, they undergo a series of morphologic changes including swelling, indentation, blebbing, and eventually disintegration into sealed globules.2–4 We have hypothesized that the process of globulization, observed with isolated fiber cells, mimics the process of supranuclear cataractogenesis, during which extensive globulization of fiber cells has been observed at the light-scattering centers.5 Such globulization of elongated cells is not unique to the lens fibers. It has also been observed in neural cells, which undergo similar disintegrative changes during calcium overload.6

To understand the role of calcium in the globulization of isolated fiber cells, we investigated the uptake of calcium by the fiber cells and its relationship to the activation of Ca⁺²-dependent proteases and globulization of single fiber cells. Our results demonstrate that in ionic media, calcium levels in the fiber cells increase significantly, followed by activation of cellular proteases leading to fiber cell globulization. Protease inhibitors protect against globulization without affecting the increase in [Ca⁺²].

MATERIALS AND METHODS

Trypsin, leupeptin, and DMSO (dimethyl sulfoxide) were purchased from Sigma (St. Louis, MO). Rhodamine 123, Syto 16, Fluo-3-AM, acetoxymethyl ester of 1,2-bis (2-amino-phenoxy) ethane N, N, N, N-tetra-acetic acid (BAPTA-AM) and t-butoxycarbonyl-leu-met-7-amino-4-chloromethylcoumarin (BOC-Leu-Met-CMAC) were obtained from Molecular Probes (Eugene, OR).

Sprague-Dawley rats (each weighing 200–250 g) were housed in accordance with institutional guidelines and were killed by a single intraperitoneal injection of sodium pentobarbital. All animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Eyeballs were removed and immersed in Ringer’s solution containing (in millimolar): 150 NaCl, 5.4 KCl, 2 MgCl₂, 2 CaCl₂, and 10 HEPES (pH 7.4), with pH adjusted with NaOH. The osmolality of the solution was 300 to 310 mOsm. Eyeballs were dissected in Ringer’s solution, and the lenses were removed with the capsules intact. The single fiber cells were isolated as described previously.2 Briefly, the lenses were incubated with HEPES-EDTA-sucrose (HES) solution containing (in millimolar): 280 sucrose, 10 Na-
EDTA, and 10 HEPS (pH 7.4), osmolarity of 300 to 310 mOsm, containing 0.6 mg trypsin/ml for 15 minutes at 33°C, after which the solution was replenished. The lenses were reincubated in fresh HES solution at 35°C for 6 minutes, and then the solution temperature was gradually increased at a rate of 0.8°C/min for 6 minutes and maintained at 37°C for an additional 3 minutes. After incubation, the lenses were washed with 5 ml of HES solution and decapsulated under a dissection microscope (SMZ2T; Nikon, Tokyo, Japan). The decapsulated lenses were transferred to a tube (Eppendorf, Fremont, CA) containing 1 ml of HES solution and were rotated gently at a rate of one revolution/sec for 20 minutes at room temperature. This procedure results in the dissociation of fiber cells, mainly from the outer cortex.6

Detection of Subcellular Organelles in the Isolated Fiber Cells

The isolated fiber cells were loaded with fluorescent dyes to label mitochondria or nuclei. Human lens epithelial cells with extended life span (HLE B-3 cells), a gift from Usha Andley (Washington University, St. Louis, MO), were used as positive standards. They were derived from an infant human lens epithelial culture by Ad12SV40 hybrid virus infection. The HLE B-3 cells were cultured in 20% fetal bovine serum in Eagle’s minimum essential medium containing 50 μg/ml gentamicin, according to previously published procedures.7 The cells were incubated for 1 hour at 37°C with 0.1 μM mitochondrial fluorescent dye, rhodamine 123, or the DNA-specific fluorescent dye Syto 16. The cells were washed with the dye-free buffer and plated on a coverslip, viewed, and photographed by microscope (Eclipse; Nikon) using Nomarski differential interference and fluorescence microscopy. Fluorescence photomicrographs were taken immediately after exposure of the cells to the excitation wavelengths, because both dyes are particularly sensitive to photobleaching. The cells were excited at 488 nm and fluorescence emission measured above 510 nm. Rhodamine 123 is positively charged and concentrates in mitochondria because of the large membrane potential in these organelles. Syto 16 concentrates in the nucleus, in that this dye specifically binds to DNA.

Measurement of Globulization Time

The isolated cells were plated on a coverslip at the bottom of a tissue bath maintained at room temperature (~23°C). In approximately 15 minutes, the fiber cells loosely attach to the coverslip. The fiber cells were superfused in the indicated solutions at a constant flow rate of 0.1 ml/min, and the changes in fiber cell morphology were monitored. In some experiments, the isolated cells were superfused in the indicated solutions at a constant flow rate of 0.1 ml/min, and the changes in fiber cell morphology were monitored. After incubation, the cells were layered on the coverslip at the bottom of the tissue chamber in different solutions. Fluorescence (F) of the fiber cell was measured at an excitation wavelength of 490 nm, and an emission wavelength of 520 nm. Maximum fluorescence (F_max) was determined by the addition of 10 μM of the ionophore A23187, and F_min was determined by measuring the fluorescence after quenching by the addition of 2.0 mM MnCl₂. The [Ca²⁺], was calculated using the following equation:

\[
[Ca^{2+}] = K_d \times \frac{(F - F_{\text{min}})}{(F_{\text{max}} - F)}
\]

K_d of 400 nM, representing the dissociation constant of Ca²⁺ bound to the dye (Fluo-3), was used for the calculations.

Measurement of Intracellular Proteolytic Activity in Single Fiber Cells

The proteolytic activity of individual fiber cells was determined by incubating the fiber cells with the protease substrate, BOC-Leu-Met-CMAC. This substrate readily permeates through biological membranes and once inside the cell, conjugates with GSH to form BOC-Leu-Met-MAC-SG.8 Cellular proteases cleave the conjugate between methionine and MAC-SG resulting in the fluorescent product, MAC-SG, which has an emission maxima at 410 nm when excited at 360 nm. The BOC-Leu-Met-CMAC was dissolved in DMSO to make a stock solution of 4.5 mM. The fibers were preincubated in 0.5 ml of HES solution containing 10 μM substrate for 5 minutes and then transferred to a circular tissue chamber, which had 3 ml of indicated solution containing 10 μM substrate. Fluorescence from a single fiber was measured using a microfluorometer built around an inverted microscope (Diaphot 300; Nikon) equipped with an epifluorescence attachment and two photomultiplier tubes.9 The fiber cells were plated on a coverslip attached to the bottom of the circular tissue chamber. The fibers were illuminated with a 150 W xenon lamp, powered by a constant current power supply (Universal Power Supply, model 68805; Oriel, Stratford, CT). The light from the lamp was collimated by a beam probe and delivered to the fiber assembly through a dichroic mirror installed in the microscope. The fiber cell was illuminated with excitation light at 360 nm, and the emission fluorescence was measured at 410 nm, using a long-pass filter. The fluorescence from the fiber cell was collected via a ×20 objective lens (CF Fluor; Nikon) and conducted through the side port of the microscope. To minimize collection of stray light, a rectangular shutter (model 85291; Nikon) was used to mask the portion of the image not occupied by the fiber cell. The masking cube was connected to a viewer (PXV; Nikon) for alignment and optical viewing of the fiber cell. The photomultiplier tubes (PMT, HC-124-05; Hamamatsu, Hamamatsu, Japan) were connected to the microscope through a beam-splitter holder. The PMTs were energized using a 12 V power supply (LPS 151; Leader Electronics, Cypress, CA). The gain of the PMT was set by adjusting the voltage on the analog-digital board to be between 500 to 900 V. The setup was used in a single-photon counting mode, and the data were acquired by a set of concatenated counters (TIP-10) using LabView software (National Instruments, Baltimore, MD). The microfluorometer was placed on a vibration-free table and covered with a dark cage to minimize interference from stray light. The protease activity was expressed as a change of fluorescence per minute. For each experiment, the change in fluorescence of single fiber cell was recorded for 30 minutes, but for all calculations of the enzyme activity only the slope of the linear increase in fluorescence was used.

Inhibition of Fiber Cell Proteolysis

The effect of the protease inhibitor, leupeptin, on the proteolytic activity of single fiber cells was tested using the fluorescence method described. Leupeptin was added to the reaction mixture to a final concentration of 0.5 mM. The intracellular calcium in the fiber cells was buffered by preincubating the fibers with 1.3 μM BAPTA-AM in HES solution for 30 minutes at 37°C. After incubation, BAPTA-AM was removed by perfusion with the indicated solution.

Statistical Analysis

All results are expressed as mean ± SEM. Statistical significance of differences between the treatment groups was determined using the t-test. Difference was considered significant at P < 0.05. Linear and
RESULTS

Detection of Subcellular Organelle in Isolated Fiber Cells

We analyzed a number of fibers for the presence of mitochondria and nuclei in the isolated fiber cells, but only two fibers each are presented in Figures 1 and 2 for mitochondria and DNA stain, respectively. The top rows in both Figures 1 and 2 show the Nomarski differential interference contrast images, and the bottom rows show the fluorescence images of the same cells. As shown in Figure 1C2 the mitochondria-specific fluorescent dye, Rhodamine 123, robustly labeled the subcellular structure that corresponds to mitochondria in human lens epithelial cells, but the fiber cells were only nonspecifically uniformly labeled (Figs. 1A2, 1B2). Similarly, in Figure 2 C2, the DNA-specific fluorescent dye Syto 16 robustly labeled the subcellular structure that corresponds to nuclei in cultured human lens epithelial cells, but only nonspecific uniform fluorescence was observed in the fiber cells (Figs. 2A2, 2B2). Based on previous studies, our results indicate that mitochondria and nuclei are labeled in Figures 1C and 2C, respectively, and the uniform labeling in Figures 1A and 1B are presumably background nonspecific labeling. Thus, we demonstrate that the fiber cells that we have used in our studies are devoid of subcellular organelles such as mitochondria and nuclei.

Single fiber cells isolated from the rat lens cortex display elongated morphology. Superfusion of these cells with Ringer’s solution containing $2 \times 10^{-3} \text{ M} [\text{Ca}^{2+}]_o$ led to a series of morphologic changes in fibers, which resulted in the formation of round, resealed globules. The globulization time of fiber cells in different solutions is given in Table 1. The fiber cells did not globulize in HES solution or Ringer’s solution with $10^{-8} \text{ M} [\text{Ca}^{2+}]_o$ for the maximal observation time of 120 minutes. In Ringer’s solution with $2 \times 10^{-3} \text{ M} [\text{Ca}^{2+}]_o$, the fiber cells globulized in approximately 25 minutes, which was delayed to approximately 50 minutes when the $[\text{Ca}^{2+}]_o$ was reduced to $10^{-6} \text{ M}$. Preincubation of fibers with the cell-permeable calcium-binding dye Fluo-3-AM followed by incubation in $2 \times 10^{-3} \text{ M} [\text{Ca}^{2+}]_o$ Ringer’s solution increased $T_g$ to approximately 35 minutes from 25 minutes in $2 \times 10^{-3} \text{ M} [\text{Ca}^{2+}]_o$ Ringer’s solution. Also, preincubation of fibers with the protease substrate, BOC-Leu-Met-CMAC, significantly delayed globulization of fiber cells in Ringer’s solution containing $2 \times 10^{-3} \text{ M}$ or $10^{-6} \text{ M} [\text{Ca}^{2+}]_o$. Addition of leupeptin (0.5 mM) to Ringer’s solution containing $2 \times 10^{-3} \text{ M} [\text{Ca}^{2+}]_o$, or buffering intracellular calcium by BAPTA-AM increased the $T_g$ to approximately 100 minutes.

The $[\text{Ca}^{2+}]_o$ of the isolated fiber cells determined at different $[\text{Ca}^{2+}]_o$ and in the presence of the protease inhibitor

**Figure 1.** Lens fibers and human cultured lens epithelial cells loaded with rhodamine 123. (A1, B1, and C1) Nomarski differential interference contrast; (A2, B2, and C2) fluorescence images. (A) and (B) are images from lens fibers, and (C) are from cultured human lens epithelial cells. (B1, B2) Two cells together. Labeling with rhodamine 125 was uniform in the lens fibers and did not appear to be associated with any intracellular organelle. There was clear labeling of the dense distribution of mitochondria surrounding the nucleus. Scale bar, 50 μM.

**Figure 2.** Lens fibers and human cultured lens epithelial cells loaded with Syto 16. Panels are arranged as in Figure 1. Labeling with Syto 16 was uniform in the lens fibers and concentrated in the nucleus in the epithelial cells. Scale bar, 100 μm (B1, B2).
leupeptin and the Ca\(^{2+}\)-chelator BAPTA are shown in Table 2. In agreement with previous reports, the [Ca\(^{2+}\)]\(_i\), in fiber cells in HES or 10 \(\times\) 10\(^{-6}\) M [Ca\(^{2+}\)]\(_o\), Ringer’s solution was approximately 100 nM. In Ringer’s solution containing 2 \(\times\) 10\(^{-3}\) M [Ca\(^{2+}\)]\(_o\), the increase was approximately two-fold. Addition of leupeptin had no significant effect on the levels of [Ca\(^{2+}\)]\(_i\), in fiber cells incubated with 2 \(\times\) 10\(^{-3}\) M [Ca\(^{2+}\)]\(_o\), Ringer’s solution.

The proteolytic activity of the isolated fiber cells, determined by measuring the increase in fluorescence was maximum in the fibers incubated with 2 \(\times\) 10\(^{-3}\) M [Ca\(^{2+}\)]\(_o\), Ringer’s solution. The dependence of the proteolytic activity on [Ca\(^{2+}\)]\(_i\), is shown in Figure 3. The proteolytic activity was 8- and 13-fold higher in Ringer’s solution containing 10\(^{-6}\) M and 2 \(\times\) 10\(^{-5}\) M [Ca\(^{2+}\)]\(_o\), respectively, than in HES solution (Table 2). No significant difference in the proteolytic activity was observed between fiber cells in HES and 10\(^{-8}\) M [Ca\(^{2+}\)]\(_o\), Ringer’s solution. Proteolysis in the fiber cells exposed to 2 \(\times\) 10\(^{-3}\) M [Ca\(^{2+}\)]\(_o\), was almost completely inhibited by leupeptin. In cells loaded with BAPTA-AM, the proteolytic activity was significantly lower than in the cells without BAPTA-AM exposed to the same [Ca\(^{2+}\)]\(_o\).

**DISCUSSION**

Accumulation of calcium in the lens has been associated with cataractogenesis. Increased calcium in the lens has been observed in more than 75% of all the cataracts examined, and inhibition of a Ca\(^{2+}\) pump by calmodulin antagonists has been shown to cause changes in lens permeability and transparency. Nevertheless, calcium is essential for maintaining the transparency of the lens, because the culture of intact lenses in the absence of calcium results in opacification. Thus, calcium homeostasis is essential for the maintenance of lens transparency. In intact lens, intracellular calcium is maintained mainly by the highly active single layer of epithelium present at the anterior surface of the lens. At the equatorial regions,

**Ca\(^{2+}\)-Dependent Protease(s) and Lens Fiber Globulization**

**TABLE 2.** The Effect of Calcium on the Protease Activity in Fiber Cells Isolated from Rat Lens Cortex

| Solution | [Ca\(^{2+}\)]\(_i\), (nM) | | |
| --- | --- | --- | --- | --- |
| | 5 Minutes | 10 Minutes | 15 Minutes | Protease Activity (Fluorescence: \(10^3\) cpm) |
| Solution 1 | | | | |
| 2 \(\times\) 10\(^{-3}\) M Ca\(^{2+}\) | ND | ND | 17.5 (7) | 0.9 \(\pm\) 0.2 (7) |
| 10\(^{-6}\) M Ca\(^{2+}\) | 191.1 \(\pm\) 22.8 (10) | 252.5 \(\pm\) 23.6 (11) | 784.5 \(\pm\) 130.6 (12) | 12.9 \(\pm\) 2.1 (12) |
| 10\(^{-9}\) M Ca\(^{2+}\) | 94.0 \(\pm\) 10.8 (6) | 128.5 \(\pm\) 12.9 (6) | 172.3 \(\pm\) 26.3 (6) | 7.8 \(\pm\) 1.7 (9) |
| Solution 2 | | | | |
| Leupeptin (0.5 mM) | 176.0 \(\pm\) 17.3 (6) | 246.9 \(\pm\) 25.3 (6) | 622.5 \(\pm\) 72.7 (7) | 1.3 \(\pm\) 0.5 (12) |
| BAPTA-AM (1 \(\mu\)g/ml) | ND | ND | ND | 2.6 \(\pm\) 0.7 (9) |
| HES Solution | ND | ND | 113.8 \(\pm\) 17.5 (7) | 0.9 \(\pm\) 0.3 (23) |

Fiber cells were isolated from the rat lens cortex, and protease activity was measured in a microfluorimeter using 10 \(\mu\)M BLMC as the substrate. [Ca\(^{2+}\)]\(_o\), in isolated fibers in HES was 90 \(\pm\) 5 nM (data not shown). Calcium measurements were made using Fluo-3-AM. Data are expressed as mean \(\pm\) SEM. Number of fibers analyzed is in parentheses. Solutions are described in Table 1. ND, not determined.

\(^*\) \(P < 0.05\) compared with the 5-minute value of the same treatment group.

\(\dagger\) \(P < 0.01\) compared with the 5-minute value of the same treatment group.
epithelial cells divide and differentiate into elongated fibers. The fibers accumulate crystallins (~33%) and gradually lose their intracellular organelles. Analysis of 10 to 12 fibers each after staining with fluorescent dyes specific for DNA and mitochondria indicated that the fiber preparation that we use for all the investigations of calcium levels, protease activity, and the globulization time did not have nuclei or mitochondria. Presumably, replacement of intracellular organelles with crystallins during maturation of fiber cells minimizes light scattering. Although in the mature lens, the highly differentiated fibers are not electrogenic, they maintain a negative potential (approximately −45 to −60 mV) by being coupled through low-resistance pathways to the epithelium. This is supported by the observation that after removal of the single anterior layer of the epithelium, the resting potential in the lens decreases to near zero. Moreover, the observation that the Ca²⁺-ATPase activity of the cortex is low, further supports the view that calcium homeostasis in the lens is maintained in part by the epithelium.

We have previously shown that single fiber cells isolated from rat lens cannot maintain [Ca²⁺]i, in an ionic media (Ringer’s solution) containing physiological levels of calcium present in the interstitial spaces of the lens. Increased [Ca²⁺]i, in isolated fibers, superfused with Ringer’s solution containing different concentrations of calcium, is associated with globulization of the elongated fibers into small sealed globules which exclude trypan blue as well as Lucifer yellow. The globulization of fiber cells appears to be mediated, in part, by the activation of chloride channels and Donnan swelling. The globules, generated in vitro, resemble those observed in the light-scattering centers of the diabetic and senile supranuclear cataracts. We find that the globules, generated from isolated fibers, have uniform electron density, similar to that observed with thin sections of intact lens cortex and have no marked inhomogeneities or high-molecular-weight protein aggregates that can scatter light. Because protein aggregates of approximately 1 million Da are required to cause light scattering, it appears likely that formation of these globules, rather than overt protein aggregation, is the underlaying cause of increased light scattering in supranuclear cataracts.

Our results show that an increase in Ringer’s solution with [Ca²⁺]i, from 10⁻⁸ to 2 × 10⁻³ M leads to an increase in fiber cell [Ca²⁺]i, with a decrease in globulization time from more than 120 minutes to less than 30 minutes. The increase in [Ca²⁺]i, was closely associated with the proteolytic activity of single fibers. Although for technical reasons it was not possible to measure calcium levels and protease activity in the same fiber cell, analysis of each one of these parameters separately in a large number of fibers provided statistically significant correlation between these parameters. An R² value of 0.897 was obtained from linear regression analysis relationship between [Ca²⁺]i (after 15 minutes of exposure to Ringer’s solution) and protease activity (Table 2). However, a single exponential fit of the relationship gave an R² value of 0.995, indicating that [Ca²⁺]i, and the protease activity were highly correlated.

One of the likely roles of increased [Ca²⁺]i, may be activation of Ca²⁺/calpain, which may be involved in fiber cell globulization. In fiber cells superfused with 2 × 10⁻³ M Ca²⁺, Ringer’s solution containing 0.5 mM leupeptin, a protease inhibitor, indicates that activation of Ca²⁺/calpain plays a critical role in the disintegration of isolated fiber cells in the media containing calcium. The globulization appears to be triggered by the increase in [Ca²⁺]i, because preloading cells with BAPTA-AM, which is cleaved inside the fiber cells by pyridine coenzyme-linked aldehyde dehydrogenase and is trapped inside cells, prevented globulization. Thus, our results clearly show that in ionic media, increased calcium entry resulting in the activation of cellular proteases leads to globulization of fiber cells. However, further investigations are required to identify the specific substrate(s) of the Ca²⁺-activated protease, which may be involved in fiber cell globulization.

In view of the similarity between disintegrative globulization of fiber cells and the light-scattering centers of supranuclear cataracts, it is tempting to speculate that chemical or physical injury to the fiber cells could result in their uncoupling from the epithelium. The uncoupled fiber cells, akin to the isolated single cells, will be destined to globulize, because of their inability to maintain intracellular calcium. In the presence of millimolar concentrations of calcium in the interstitial fluid, the [Ca²⁺]i, would increase in the uncoupled fibers resulting in the activation of proteases which would then cleave structural proteins to form ressealed globules. By enhancing light scattering, these globules would cause punctate opacity in the cortex, generally observed in early stages of supranuclear cataract.

References