In Vitro Large-Wound Re-endothelialization
Inhibition of Centrosome Redistribution by Transient Inhibition of Transcription After Wounding Prevents Rapid Repair

David S. Ettenson, Avrum I. Gotlieb

Rapid, efficient re-endothelialization of large wounds is characterized by a specific sequence of cytoskeletal events that occur after wounding. Wounds 1.5 mm wide were created down the middle of confluent porcine aortic endothelial monolayers to study regulation of repair. The wounded cultures were incubated for short periods with cycloheximide or actinomycin D to test the hypothesis that transient inhibition of translation and transcription at the time of wounding disrupts rapid repair by interfering with centrosome redistribution to the front of the cell, an early event associated with cell migration. Although centrosome reorientation did not occur when protein synthesis was inhibited with 20 μg/mL cycloheximide for 1 hour before and for up to 4 hours after wounding, reorientation did occur by 2 hours after cycloheximide was washed out. The times taken for the wound to close for cycloheximide-treated and control cells did not differ (60±1.1 vs 60±0.8 hours). When transcription was inhibited with 0.25 μg/mL actinomycin D for 1 hour before and for 1 hour after wounding, re-endothelialization was dramatically reduced. The time taken for the wound to close was almost five times longer (288±5.3 hours) than for control cells. The cells moved very slowly, maintaining a flattened, spread-out shape, as opposed to being elongated. The centrosomes did not reorient to the front of the cell throughout the entire period. However, addition of actinomycin D for 2 hours when centrosomes had already moved to the front of the cells (4 hours after wounding) did not reduce subsequent wound repair (60±1.3 hours). This study supports our hypothesis that centrosome redistribution is essential for efficient wound repair and suggests that redistribution is regulated by transcription of essential gene(s) that is induced immediately after wounding by an unknown short-lived signal. Two possible signals are the loss of cell contact and/or a soluble substance released from the cells at the time of wounding. When the signal is unable to induce transcription, dysfunctional repair occurs by a very slow centrosome-independent process. (Arterioscler Thromb. 1993;13:1270-1281.)

KEY WORDS • centrosome • microtubules • microfilaments • actinomycin D • endothelial cells • cycloheximide • wound

Under physiological conditions, an intact endothelial monolayer, which is required for the normal functioning of the blood vessel wall, is probably maintained by the presence of cellular processes that regulate rapid endothelial repair of very small wounds, some involving a single cell. However, in some pathobiological states, such as complicated atherosclerotic plaques, atherectomy and angioplasty, and saphenous vein bypass grafts, large areas of denudation are present and rapid efficient repair is essential to re-establish a structurally intact endothelium. The regulation of this complex repair, however, is not well understood.

We do know that cytoskeletal fiber systems are important in regulating endothelial cell (EC) shape and migration during the repair of large wounds. Microfilaments appear to play a role in the force-generation "machinery" of the cytoskeleton and in cell substratum and cell-cell adhesion, whereas the centrosome and its associated microtubules play an important role in directional cell migration.

We have previously shown that in uninjured confluent monolayers the ECs are surrounded by a dense peripheral band (DPB) of actin microfilament bundles and contain a few central microfilament bundles. The centrosomes are normally found randomly distributed around the nucleus. Using large- and small-wound models, we have shown that re-endothelialization is regulated by a specific sequence of changes that involves the centrosomes and their associated microtubules and the microfilaments. After wounding of an endothelial monolayer, adjacent ECs rapidly extrude lamellipodia into the denuded area. This occurs independently of centrosomes or microtubules, and the wounds of one to four cells are re-endothelialized by this method. These are likely to be the processes at work under normal physiological conditions. However, if repair is incomplete, which occurs in larger wounds, a second set of events occurs that is characterized by...
centrosome redistribution toward the front of the cell and elongation of the EC in preparation for cell translocation. DPB breakdown ensues, central microfilaments become more prominent, and directed migration occurs to re-endothelialize the wound. In these larger wounds, centrosome redistribution is an early and important feature of repair.

In this study, we designed experiments to test the hypothesis that disruption of critical cytoskeletal events in the early stages of wound repair results in the disruption of efficient wound repair. We disrupted putative early regulatory events that occur after wounding by inhibiting protein synthesis and transcription using cycloheximide and actinomycin D, respectively. Inhibition of transcription around the time of wounding resulted in failure of the centrosomes to redistribute to the front of the cells even long after the actinomycin D was removed and transcription was re-established. This had profound effects by seriously delaying wound repair. We also found that repair could occur by a centrosome-independent pathway that was, however, a much less efficient, slower process.

Methods

Cell Cultures

ECs were harvested from slaughterhouse porcine aortas by using the collagenase enzyme dispersion method described previously. Cultures were grown in medium M199 supplemented with 5% fetal bovine serum (FBS), 50 units/ml penicillin, 50 µg/mL streptomycin, and 0.25 µg/mL Fungizone (M199 and 5% FBS). Cultures were fed every 2 days. Subculturing was performed on confluent cultures using 0.05% trypsin and 0.02% EDTA. Cells from passages 2 through 4 were used in the wounding experiments. All tissue-culture reagents were obtained from GIBCO Laboratories (Grand Island, NY).

Wounding

ECs were seeded in 60-mm dishes containing 22 x 40-mm sterile glass coverslips. Two days after the culture reached confluency, a 1.5-mm wound was made down the middle of the monolayer by completely removing the cells in this region with a flat-edged Teflon spatula. The time of wounding was designated as time 0 hours. The wound area was marked by small scratches made with a sterile diamond pencil. The dishes were analyzed to determine the percentage of cells with a centrosome in each EC participating in wound repair was classified as being "Toward," "Middle," or "Away" with respect to the nucleus and the front of the cell in relation to the wound edge or the direction of cell migration. A centrosome classified as Toward was located between the nucleus and the side of the cell closest to the wound edge, whereas one that was classified as Away was located between the nucleus and the side of the cell facing away from the wound. A centrosome in the Middle was located along the side of the nucleus. One hundred cells, on each side of the wound, were analyzed to determine the percentage of cells with centrosomes in each of the three locations.

Incubation of Cells With Actinomycin D and Cycloheximide

To determine if de novo synthesized mRNA and protein synthesis were required for wound repair, EC monolayers were preincubated for 1 hour at 37ºC with 0.25 µg/mL actinomycin D (Sigma) or 20 µg/mL cycloheximide (Sigma). The monolayer was then wounded and incubated at 37ºC with actinomycin D or cycloheximide. One or 4 hours later the drug was removed by washing the monolayers four times with PBS containing Ca²⁺/Mg²⁺. Fresh M199 and 5% FBS were added to the cells. In some studies actinomycin D was added 4 hours after wounding and washed out 2 hours later. All cultures were fed every 2 days with M199 and 5% FBS for the duration of the experiment.
[3H]Uridine Incorporation Studies

To ensure that actinomycin D reversibly inhibited RNA synthesis, [3H]uridine incorporation studies were performed. EC cultures were grown in 12-well tissue-culture plates and were used 2 days after they reached confluency. Some cultures were incubated with 0.25 μg/mL actinomycin D for 1 hour before and 1 hour after wounding before being washed out. Both nontreated and actinomycin D–treated nonwounded and wounded cultures were incubated with 5 μCi/mL [5,6-3H]uridine (specific activity=44 mCi/mmol; ICN Immunobiologicaals) during the final 30 minutes of incubation. The cultures were washed three times with PBS containing Ca^{2+}/Mg^{2+} to remove nonincorporated [3H]uridine. The cells were detached by incubation with 1 mL trypsin for 20 minutes and collected in centrifuge tubes. The wells were washed with 1 mL PBS containing Ca^{2+}/Mg^{2+} that was added to the tubes. The cells were then precipitated with 1 mL cold 10% trichloroacetic acid (TCA) at 0°C for 30 minutes, followed by centrifugation for 10 minutes at 500g. The pellets were washed three times with cold 10% TCA. The residues were solubilized in 1 mL of 1N NaOH. Radioactivity was determined by adding 100-μL aliquots into 5 mL scintillation fluid (Cytoscint ES; ICN Immunobiologicaals). The vials were counted by a liquid scintillation counter.

Metabolic Labeling Studies

To determine the extent of inhibition of protein synthesis by cycloheximide and its reversibility after washing, metabolic labeling studies were performed. ECs were plated into 12-well tissue-culture plates and used 2 days after they reached confluency. The cultures were washed four times with prewarmed PBS containing Ca^{2+}/Mg^{2+} to remove any traces of FBS. The cultures were then incubated with methionine/cysteine-free RPMI medium (ICN Immunobiologicaals) containing 0.1% bovine serum albumin (BSA) for 15 minutes at 37°C. It was then removed and replaced with fresh methionine/cysteine-free RPMI medium containing 0.1% BSA, trans 35S-label (100 μCi/mL; specific activity=1192 mCi/mmol; ICN Immunobiologicaals) was added to nontreated control cells as described in "Methods." Endothelial cultures were treated with 20 μg/mL cycloheximide for 1 hour before and for 1 hour after wounding (−1h−→+1h) or 4 hours after wounding (−1h−→+4h) before being washed out. The radioactivity per 1×10^5 cells was determined starting 1 hour after wounding. Protein synthesis was inhibited in the presence of cycloheximide but resumed within 2 hours after cycloheximide washout. Values represent mean±SEM (n=9).

Results

Effects of Protein Synthesis Inhibition on Endothelial Repair

To determine if de novo synthesis of protein early after wounding was required for efficient repair, cells were incubated with 20 μg/mL cycloheximide for a total of 2 hours, 1 hour before and 1 hour after wounding. This dose of cycloheximide reduced the level of protein synthesis to 2% of control levels; however, protein synthesis resumed after washout of the cycloheximide (Fig 1). This treatment had no effect on the rate of wound closure (Fig 2). The wounds closed in 60±1.3 hours at an average rate of 21.8±3.4 μm/h compared to control wounds. A 1-hour washout of cycloheximide allowed repair to be restored to control levels, as determined by the reendothelialization index (Fig 2). Statistical analysis by analysis of variance. If a significant difference was seen, then a Newman-Keuls test was performed to determine which treatments were significantly different from each other.15

Statistical Analysis

The times of wound closure and the centrosome positions of the different treatment groups were compared by an analysis of variance. If a significant difference was seen, then a Newman-Keuls test was performed to determine which treatments were significantly different from each other.15
FIG 3. Bar graphs showing redistribution patterns of centrosomes in the first row of cells during wound repair in the absence (A) or presence of 20 μg/mL cycloheximide for 1 hour before and for 1 hour (B) or 4 hours (C) after wounding. The position of the centrosome in each cell along the wound edges was determined and expressed as a percentage of the total number of cells. Values represent mean±SD (n=9). In both cases, the centrosomes did not reorient in the presence of cycloheximide but reached control levels of 80% toward the wound by 2 hours after the drug was washed out. The centrosomes began to randomize after the wound closed in all cases. The c-labeled arrow indicates time of wound closure.

with closure in 60±0.8 hours at an average rate of 22.7±5.7 μm/h for control cells. However, there was a slight delay in the rate of centrosome redistribution in the presence of cycloheximide (Fig 3B). During the hour after wounding, in the presence of cycloheximide, there were still many centrosomes facing away from the wound edge (Fig 3B) compared with controls (Fig 3A). However, by 2 hours after the cycloheximide was washed out, the centrosomes reoriented to control levels and then followed the same distribution pattern as control cells (Fig 3A and 3B). To better demonstrate the effect of cycloheximide on the delay of centrosome reorientation, cultures were incubated with cycloheximide for a total of 4 hours after wounding before being washed out. When this was done, the delay in centrosome redistribution was clearly seen (Figs 3C and 4B). The centrosomes did not reorient until the cycloheximide was removed, and they reached control levels 2 hours after washout (Figs 3C and 4D). The microfilaments were not affected by this treatment (Fig 4A, C, E, and G). The overall rate of wound closure was not affected by the 4-hour incubation with cycloheximide (Fig 2), as the cells moved by flattening out, but they did not elongate when they moved. When the actinomycin D was removed, 1 hour after wounding, the DPBs were not very prominent (Fig 7A), but they were more prominent 6 hours after wounding (Fig 7C). The microtubules appeared normal for the first 6 hours after wounding (Fig 7B and 7D). By 24 hours the cells contained a more pronounced DPB with relatively few central microfilament fibers (Fig 7E). The actin filaments displayed a punctate staining pattern (Fig 7F) that was less pronounced by 96 hours after actinomycin D had been washed out (Fig 7H). However, the centrosomes...
Actinomycin D was added for either 1 hour before and for 1 hour after wounding. Cells were double stained to localize F-actin (A, C, E, G) and tubulin (B, D, F, H). Cells are shown after wounding at the following times: 4 hours (A, B), 6 hours (C, D), 24 hours (E, F), and 132 hours (G, H). In A through F one wound edge is present on the right-hand side; the other side appeared similar. During the 4 hours that the cells were incubated with cycloheximide the centrosomes did not reorient to the front of the cells (B), but by 2 hours after washout 80% of the centrosomes faced toward the wound edge (D) (data not shown). By 24 hours after wounding the cells were elongated, and the microfilaments were oriented parallel to the direction of migration (E). By 132 hours the monolayer had closed, and the cells were cobblestone in shape, as they were before wounding (G, H). Black arrows indicate centrosomes; white arrowhead, middle of wound. Bar=10 μm.

were randomly distributed around the nucleus throughout the entire experiment and did not reorient (Fig 8A). By 144 hours, the microfilament bundles at the periphery became less pronounced and the central fibers were more evident (Fig 7I). In addition, the cells appeared much larger (Fig 7I and 7J). With time the cells reduced in size, and when the wound closed, the cells did not pile up on each other but were elongated (Fig 7K and 7L). Forty-eight hours after closure the monolayer assumed a cobblestone appearance (Fig 7M and 7N). The distribution of microfilaments appeared more like control monolayers (Fig 7M), but the microtubule system was still fairly disrupted (Fig 7N). In addition, the speed of cell movement appeared to depend on the presence of an intact microtubule system. When the microtubules were reduced during the first 72 hours, the cells moved at an average speed of only 2.3±0.6 μm/h. However, on reappearance of the microtubule system by 96 hours, the speed of movement increased to 6.7±1.3 μm/h, which was still, however, significantly slower than control cells (P<.001).

Whereas inhibition of protein synthesis during the first hour after wounding had only a transient effect on the repair process, inhibition of transcription by actinomycin D dramatically altered this process. Although transcription was inhibited for only 1 hour after wounding, its effects were long-lasting. It affected the way the cells moved and their speed of movement. It also affected the distribution of microfilaments and centrosomes and their associated microtubules. When lower concentrations of actinomycin D were used that did not totally inhibit transcription, the repair process was not altered. If higher concentrations were used, then the cells began to detach from the coverslips within 2 to 4 hours (data not shown). Thus, the effect of actinomycin D on the repair process was dose related and was dependent on the degree of inhibition of transcription.

When 0.25 μg/mL actinomycin D was added at 4 hours after wounding (well after the centrosomes had moved toward the front of the cells) and then washed out 2 hours later, cell migration and wound closure occurred normally, with closure occurring in 60±1.3 hours (Fig 6). The microfilament and microtubule systems appeared normal (Fig 9) and the centrosomes redistributed in a manner similar to control cells (Figs 8B and 9). By the end of this 2-hour incubation, the level of [3H]uridine incorporation was reduced to 2% of control levels (Fig 5). Thus, as long as centrosome redistribution and probably other unknown early events had already occurred after wounding, inhibiting RNA synthesis had no effect on the repair process.

Discussion

Large-wound re-endothelialization is a two-step process. The first step occurs independently of centrosome redistribution. This step is characterized by EC extrusion of lamellipodia into the denuded area. The second

![Figure 4](http://atvb.ahajournals.org/)

**Figure 4.** Facing page. Photomicrographs of confluent endothelial cell cultures treated with 20 μg/mL cycloheximide for 1 hour before and for 4 hours after wounding. Cells were double stained to localize F-actin (A, C, E, G) and tubulin (B, D, F, H). Cells are shown after wounding at the following times: 4 hours (A, B), 6 hours (C, D), 24 hours (E, F), and 132 hours (G, H). In A through F one wound edge is present on the right-hand side; the other side appeared similar. During the 4 hours that the cells were incubated with cycloheximide the centrosomes did not reorient to the front of the cells (B), but by 2 hours after washout 80% of the centrosomes faced toward the wound edge (D) (data not shown). By 24 hours after wounding the cells were elongated, and the microfilaments were oriented parallel to the direction of migration (E). By 132 hours the monolayer had closed, and the cells were cobblestone in shape, as they were before wounding (G, H). Black arrows indicate centrosomes; white arrowhead, middle of wound. Bar=10 μm.

![Figure 5](http://atvb.ahajournals.org/)

**Figure 5.** Line graph showing inhibition of transcription by 0.25 μg/mL actinomycin D (AD) determined by [3H]uridine incorporation and expressed as a percentage of [3H]uridine uptake by nontreated control cells as described in "Methods." Actinomycin D was added for either 1 hour before and for 1 hour after wounding (−1h−→+1h) and was then washed out, or it was added 4 hours after wounding and was washed out 2 hours later (+4h→→+6h). The time of wounding was designated as time 0. Within 1 hour after addition of actinomycin D in both experiments, the level of transcription was significantly reduced. However, 24 hours after removing the drug, the level of transcription approached control rates. Values represent the mean±SEM (n=10).

![Figure 6](http://atvb.ahajournals.org/)

**Figure 6.** Line graph showing re-endothelialization indices for cultures treated with 0.25 μg/mL actinomycin D (AD) for either 1 hour before and for 1 hour after wounding (−1h−→+1h) or added 4 hours after wounding and washed out 2 hours later (+4h→→+6h). The rate of re-endothelialization was significantly retarded by the −1h−→+1h actinomycin D treatment but was unaffected by the +4h→→+6h treatment. n=9. PBS indicates phosphate-buffered saline.
step is dependent on centrosome redistribution to the front of the cells, which is followed by DPB breakdown and cell elongation and subsequent migration. Our studies indicated that the switch from the first to the second step is dependent on de novo transcription around the time of wounding (Fig 10A). If these mRNAs are not synthesized then the centrosome-dependent pathway is not activated. Re-endothelialization
FIG 7. Photomicrographs of confluent endothelial cell cultures treated with 0.25 μg/mL actinomycin D for 1 hour before and for 1 hour after wounding. Cells were double stained to localize F-actin (A, C, E, G, I, K, M) and tubulin (B, D, F, H, J, L, N). The cells are shown after wounding at the following times: 1 hour (A, B), 6 hours (C, D), 24 hours (E, F), 96 hours (G, H), 144 hours (I, J), 288 hours (K, L), and 336 hours (M, N). In A through J one wound edge is present on the right-hand side; the other side looked similar. Twenty-four hours after washing out the actinomycin D, the cells had a more pronounced dense peripheral band and fewer central microfilament fibers (E), and the microtubule system was disrupted (F). However, with time the microtubule system (H) and the central microfilaments (I) returned. As the central microfilaments became more prominent, the cells increased in size (I). However, by the time the wound had closed, the cell size and the cytoskeleton looked more like control cells (K, L), although the microtubule system was still partially disrupted (L). At the time of closure (K, L) the cells were elongated. The monolayer returned to a normal cobblestone pattern 48 hours after closure (M, N). Black arrows indicate centrosomes; white arrowheads, middle of wound. Bar=10 μm.

is still able to occur by extending the first step; however, repair occurs in a much less efficient manner, which is schematically summarized in Fig 10B. Once centrosomes have redistributed, transient inhibition of transcription does not delay repair. Transient inhibition of protein synthesis at the time of wounding does temporarily delay redistribution, but because transcription is not disrupted, washout of protein synthesis
inhibitors allows for the transcribed message to become translated into protein. Our results also suggested that centrosomal redistribution to the front of the cell is not simply a response to the presence of an empty space but requires the synthesis of certain mRNAs and their subsequent proteins.

Immediately after wounding, the DPB was intact; however, within 3 hours the DPB began to disappear in the cells of the leading edge but was still present in cells away from the wound edge. The DPB was lost in all migrating cells. The DPB did not reappear until 24 hours after wound closure. Similar findings were found with cycloheximide; however, in the reduced migration of centrosome-independent wound closure, the DPB was present for the first 144 hours. The cells were not elongated and central microfilaments were not increased. This distribution of the microfilaments was consistent with very slow migration. After 144 hours the cells elongated, and the microfilament distribution changed slowly. The DPB remained reduced, and central microfilaments were more prominent than before.

Neither the nature of the genes involved nor the signal that activates them is known at present. Possible gene candidates include those that regulate the synthesis of microtubule-associated protein(s), which may be required for microtubule reorganization and centrosome redistribution, and actin binding proteins, which are needed for microfilament reorganization. A possible signal is basic fibroblast growth factor (bFGF), which has been shown to be released from ECs after wounding and which enhances endothelial migration. In our wound model after wounding but not at later time points (data not shown). This growth factor, after binding to its receptor on the EC, may induce the necessary gene transcription needed for efficient wound repair to occur. Further studies are required to explore this possibility.

This is the first time we have been able to inhibit centrosome redistribution without using a drug that disrupts microtubules. The presence of an intact microtubule system is essential for centrosome redistribution, but it does not initiate centrosome redistribution on its own. This was demonstrated when the centrosomes of cells treated with actinomycin D immediately before and after wounding did not reorient, even though the microtubules were only transiently disrupted. The centrosomes did not redistribute even when the microtubules were intact at 96 hours and cell movement was under way. Disruption of microtubules at the time of wounding by colchicine inhibits centrosome redistribution by both disrupting the microtubule system re-forms and the centrosomes move toward the front of the cells. This suggests that the mRNA(s) required for centrosome redistribution is formed even when microtubules are disrupted.

Using a corneal endothelium organ culture wound model, it was found that actinomycin D treatment for 15 minutes after injury prevented cell migration. These cells contained central microfilaments, but the microtubule system was disrupted. However, if actinomycin D was added 24 hours after injury, cell migration was not inhibited. Although these results are consistent with our findings, there were some differences. The corneal ECs did not migrate for a period of 72 hours after the 15-minute incubation with actinomycin D, whereas our findings were due to different sensitivities that corneal and aortic ECs have toward actinomycin D.

Although centrosomes reorient to the front of the cell during migration and repair, in some cell types there are exceptions, especially when external conditions are altered. The position of the centrosome determines the direction of cell movement in ECs in vitro, in aortic organ cultures, in aortas in vivo, and in corneal endothelium. It has also been shown to be located in the front of migrating amoebas, in 3T3 cells, and in fibroblasts. The centrosome has been reported to be behind the nucleus in randomly migrating vegetative amoebas. In chemotactically migrating human neutro-
FIG 9. Photomicrographs of confluent endothelial cell cultures treated with 0.25 μg/mL actinomycin D added 4 hours after wounding and removed 2 hours later. Cells were double stained to localize F-actin (A, C, E) and tubulin (B, D, F). The cells are shown after wounding at the following times: 6 hours (A, B), 24 hours (C, D), and 132 hours (E, F). In A through D one wound edge is present on the right-hand side; the other side looked similar. After the actinomycin D was washed out, the microfilaments looked normal (A), the microtubule system was intact (B), and the centrosomes faced toward the wound edge (B). Twenty-four hours after wounding the cells were elongated, the microfilaments were oriented parallel to the direction of migration (C), and the centrosomes still faced toward the wound edge (D). The wound closed in 60 hours, and by 132 hours the monolayer resumed its cobblestone appearance (E, F). Black arrows indicate centrosomes. Bar=10 μm.

When Dictyostelium discoideum aggregate on agar plates, most of the centrosomes are found behind the nucleus. In cells aggregating under submerged conditions, the centrosomes are randomized around the nucleus. In chemotactically migrating cells, most of the centrosomes are in front of the nucleus. Thus, in Dictyostelium discoideum the position of the centrosome is dependent on the conditions of migration and the nature of cell-cell adhesion. In a study that used chicken embryo fibroblasts, it was found that the centrosomes of cells grown on glass coverslips reoriented in the direction of cell migration, whereas...
centrosomes of cells submerged in collagen gels remained randomized around the nucleus. These studies on Dictyostelium discoideum and fibroblasts suggest that polarization of the centrosome is not required for directional migration in three-dimensional gels. However, large-vessel ECs differ from fibroblasts because the cells migrate on a substratum that defines a luminal and abluminal surface of the cell, and the cells migrate only in a two-dimensional plane. Thus, migration on a luminal surface, which occurs in ECs, may be regulated by different cytoskeletal processes.

In summary, re-endothelialization of large wounds is a two-step process, and our studies indicated that the switch from the first to the second step is dependent on de novo transcription around the time of wounding. This transcription either directly or indirectly regulates centrosome redistribution, which is an important initiator of rapid, efficient re-endothelialization. If these mRNAs are not synthesized, the centrosome-dependent pathway is not activated. Re-endothelialization can still occur by extending the first step, but the repair occurs much less efficiently. If only protein synthesis is inhibited at the time of wounding, repair will occur normally once the inhibitor is removed. It is possible that wounding itself is a signal, due to loss of cell-cell contact in the cells at the wound edge. Although the lack of cell-cell contact persists during the slow repair, centrosome redistribution does not occur even though transcription has been re-established to normal levels. It is thus more likely that wounding either liberates a short-lived signal or makes the cells more responsive to an existing agent that activates a signal transduction pathway to induce the required transcription to regulate centrosomal redistribution and the initiation of rapid re-endothelialization.
Acknowledgments

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