Cells in multicellular organisms are under constant mechanical stress, and often the plasma membrane (PM) is compromised. Fortunately, there is a vigorous repair mechanism that rapidly (within seconds) reseals the wound site by fusion with an internal membrane patch. Downstream events, remodeling of the injury site and forming replacement PM, must be carried out quickly (within minutes) if a cell is to survive multiple sequential injuries. The repertoire of proteins required to repair breaks (the PM repairome) is one of the major unknowns in this area of research. As an initial approach to defining the PM repairome, a cell surface biotinylation protocol was developed to identify intracellular proteins that become exposed at the site of reversible PM injury. It is likely that at least some of these proteins are important mediators of repair. These initial studies led to a surprising finding, namely the identification of some nuclear and endoplasmic reticulum resident proteins transiently exposed at the surface of cells that ultimately recovered from PM damage. Thus, in reversible mechanical damage to the PM, underlying cellular structures may also be injured, and will also require mechanisms for repair. Other proteins at wound sites were previously identified docking partners for pathogenic bacteria and viruses (vimentin and nucleolin), or found to be upregulated and exposed on the surface of cancer cells (nucleolin and nucleophosmin-1). The new information from these studies may lead to development of novel antimicrobial and antineoplastic drugs.

Any investigator who has utilized microinjection or electroporation to introduce macromolecules into living cells will appreciate that the plasma membrane (PM) can withstand substantial insult before the cell eventually succumbs to death pathways. What is not as widely appreciated is the knowledge, gained over many years now, that cells in living tissues also undergo significant reparable PM damage under normal physiologic conditions. Recently, muscular dystrophies caused by mutations in the protein dysferlin were shown to be the result not of muscle PM (sarcolemma) weakness, but of compromised repair. Given the potentially catastrophic consequences of failure to rapidly repair PM breaks, and the emerging complexity of the system(s) required for this to happen, it seems likely that future studies will recognize compromised PM repair as a contributing factor in other pathologic conditions.

The little that is known about PM repair has focused on the primary, cell life-saving event: salvaging the electrochemical gradient across the PM. However, even this is more complex than sometimes appreciated. For cells permeabilized by electroporation, it seems that there is a rapid phase of recovery, presumably stopping the loss of cellular constituents and entry of calcium that threatens life, followed by a slower phase that finally restores complete membrane barrier function. Rapid repair of mechanically damaged PM, measured in seconds, involves calcium-dependent fusion of an internal membrane patch at the wound site, or perhaps, in the case of small wounds (<1 μm), a direct closing of the PM around the hole. The latter process may be driven, in part, by the thermodynamically favorable self-sealing of broken phospholipid membrane sheets. However, large breaks require remodeling.
of the cortical actin cytoskeleton to facilitate membrane patching. Resolution of repair appears to involve exocytosis, followed by endocytosis, and other long-term steps (requiring several minutes). Final steps may be facilitated by formation of a contractile ring surrounding the wound, and allowing a platform for neo-synthesis of cortical cytoskeleton. The studies demonstrating a wound-associated contractile ring have utilized oocytes as the primary model system. Whether a similar process occurs in somatic cells remains to be established. Not surprisingly, repair of damaged PM relies on proteins known to mediate membrane fusion, including SNAREs and synaptotagmin.

**A PM Wound Proteome**

Discovery of the proteins required for PM repair (the PM repairome) would be a major advance, setting the groundwork for establishing protein binding partners and mechanisms of repair. Toward this goal, a PM wound proteome has been identified, primarily in fibroblast-like cell lines. Adherent tissue culture cells were scraped free from substratum, a process known to produce reversible PM damage. A cell impermeant biotinylation reagent (maleimide-PEG₂-biotin, MPB) was used to label proteins at membrane rupture sites. MPB is selective for cytoplasmic proteins, which, unlike most extracellular proteins, have reduced cysteine side chains. Proteins involved in repair should be present at newly generated membrane breaks. Therefore it is likely that major repair proteins would be a subset of the PM wound proteome. In fact, one of the proteins identified by this method was annexin A1, which has been shown to accumulate at membrane damage sites and appears to function in repair.

A relatively small set of PM wound-associated proteins was reproducibly identified in many experiments. These included cytoskeletal proteins (vimentin, caldesmon), endoplasmic reticulum (ER) resident proteins (ERp57, HSP47) and nuclear proteins (lamin A/C, nucleophosmin-1). Importantly, transient extracellular exposure of these proteins did not lead to death for the majority of injured cells, as shown by numerous control experiments. The finding that luminal ER proteins and nuclear proteins were among the wound proteome highlighted the impact of PM damage on internal structures. Elements of the ER are closely associated with the PM (junctional ER), as are nuclei in many cell types, and it seems reasonable that these would be damaged upon scraping cells from substratum. These observations suggest that cells have mechanisms that allow repair of mechanical damage to intracellular organelles.

**Identification of Nucleolin as a Wound-Associated Protein**

MPB was shown to label major members of the wound proteome. However, an amine-selective biotinylation reagent also detected several proteins that were not labeled by MPB, including one migrating at approximately 80–90 kDa on SDS-PAGE. This protein has now been identified as nucleolin, by tandem mass spectrometric analysis (Fig. 1). Like vimentin, nucleolin possesses only a single cysteine residue in its structure. However, unlike vimentin, it is not a major protein detected at wound sites by MPB labeling. Perhaps the nucleolin cysteine residue is sterically shielded from MPB. Nucleolin is best known as a major nucleolar protein. However, in keeping with the observation that PM wound proteome proteins have previously been demonstrated to function outside cells, some nucleolin is associated with the extracellular face of the PM. Like extracellular vimentin, cell surface nucleolin is a docking protein for bacterial pathogens and viruses.

![Figure 1. Identification of nucleolin as a PM wound-associated protein. SV-40 transformed mouse embryonic fibroblasts were scraped, wound-associated proteins were labeled with sulfo-NHS-LC-biotin (Pierce) and biotinylated proteins were isolated. Right lane: scraped cells. Middle lane: non-scraped cell control. Left lane: Invitrogen See-Blue plus 2 protein standards. Protein bands were excised and submitted for tandem mass spectrum identification.](image-url)
is interesting that many pathogens have selected two PM wound-associated proteins for targeting to cells. Is it possible that the presence of these proteins coincides with a weakened cell structure that facilitates invasion? If so, more studies on wound-associated proteins may lead to novel antimicrobial strategies.

Like nucleophosmin-1, another previously identified PM wound proteome protein, cell surface nucleolin is upregulated in various cancers. Migrating cancer cells are likely to encounter stresses that damage PM during their travels through the extracellular matrix. MV3 melanoma cells have been shown to shed PM fragments, called matrix vesicle particles, during migration through a three-dimensional culture matrix. The matrix vesicle particles are similar in constitution to the PM fragments released when attached mouse fibroblasts are scraped from culture dishes. It is tempting to speculate that the increased PM damage, reflected in release of matrix vesicle fragments, may be responsible for the large amounts of surface nucleolin on cancer cells. If so, other proteins exposed upon reversible PM damage, especially those found to be important for repair, should be excellent, readily druggable targets for development of novel antineoplastic agents.

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