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Annexin A2 is involved in Ca^{2+}-dependent plasma membrane repair in primary human endothelial cells

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ABSTRACT

Many cells in an organism are exposed to constant and acute mechanical stress that can induce plasma membrane injuries. These plasma membrane wounds have to be resealed rapidly to guarantee cell survival. Plasma membrane resealing in response to mechanical strain has been studied in some detail in muscle, where it is required for efficient recovery after insult. However, less is known about the capacity of other cell types and tissues to perform membrane repair and the underlying molecular mechanisms. Here we show that vascular endothelial cells, which are subject to profound mechanical burden, can reseal plasma membrane holes inflicted by laser ablation. Resealing in endothelial cells is a Ca^{2+}-dependent process, as it is inhibited when cells are wounded in Ca^{2+}-free medium. We also show that annexin A1 (AnxA1), AnxA2 and AnxA6, Ca^{2+}-regulated membrane binding proteins previously implicated in membrane resealing in other cell types, are rapidly recruited to the site of plasma membrane injury. S100A11, a known protein ligand of AnxA1, is also recruited to endothelial plasma membrane wounds, albeit with a different kinetic. Mutant expression experiments reveal that Ca^{2+} binding to AnxA2, the most abundant endothelial annexin, is required for translocation of the protein to the wound site. Furthermore, we show by knock-down and rescue experiments that AnxA2 is a positive regulator of plasma membrane resealing. Thus, vascular endothelial cells are capable of active, Ca^{2+}-dependent plasma membrane resealing and this process requires the activity of AnxA2.

1. Introduction

To survive and to maintain tissue integrity, several types of cells have been shown to be able to repair (or reseal) plasma membrane holes that can be as large as several μm^2 [1–3]. Such holes can form in different ways. In infectious scenarios, membrane wounds can be inflicted by pore-forming toxins that are inserted into the plasma membrane [4]. In the course of repair, such wounds can be physically eliminated by endocytosis or the shedding of microparticles containing the pore [5]. Membrane ruptures that occur in non-infected cells independent of toxin action are usually the consequence of mechanical stress. This is particularly pronounced in skeletal muscle, where membrane damage is observed in up to 20% of muscle fibers during normal exercise, and is increased in diseases such as Duchenne muscular dystrophy [6]. Although differences are likely to exist between the repair mechanisms of pore induced and physical membrane wounds, both require Ca^{2+} entering through the plasma membrane hole as a crucial initiator of resealing. In the case of mechanical wounds, published evidence suggests that the increase in intracellular Ca^{2+} induces the formation of a membrane patch underneath the site of injury, most likely through the recruitment and homotypic fusion of internal membranes. This patch then fuses with the plasma membrane, thereby replacing the hole with a new continuous membrane [3]. However, alternative mechanisms of membrane repair have been proposed and shown to occur in certain scenarios. These include endocytic retrieval of damaged membrane and ESCRT-dependent shedding of membrane vesicles containing the ruptured site [7–9]. Most likely, the mechanism chosen depends on the type and size of membrane wound and could differ between cell types, but in any case is likely to also involve a Rho-GTPase-driven remodeling of the cortical actin cytoskeleton [10].

Despite the fundamental importance of membrane repair for cell survival and tissue integrity, little is known about underlying molecular mechanisms und components of the resealing machinery. By analyzing sarcolemma repair and focusing on proteins altered in patients suffering from muscular dystrophies, dysferlin, a member of the ferlin protein family containing Ca^{2+}-sensing C2 domains, was identified. Dysferlin is mutated in limb-girdle muscular dystrophy type 2B and Miyoshi myopathy, and experiments involving cultured cells as well as developing zebrafish embryos implicate the protein as a Ca^{2+}-regulated fusogen in the course of exocytotic events occurring during membrane resealing [11–13]. Several dysferlin interacting proteins have been identified, among them two members of the annexin (Anx) family of Ca^{2+} and

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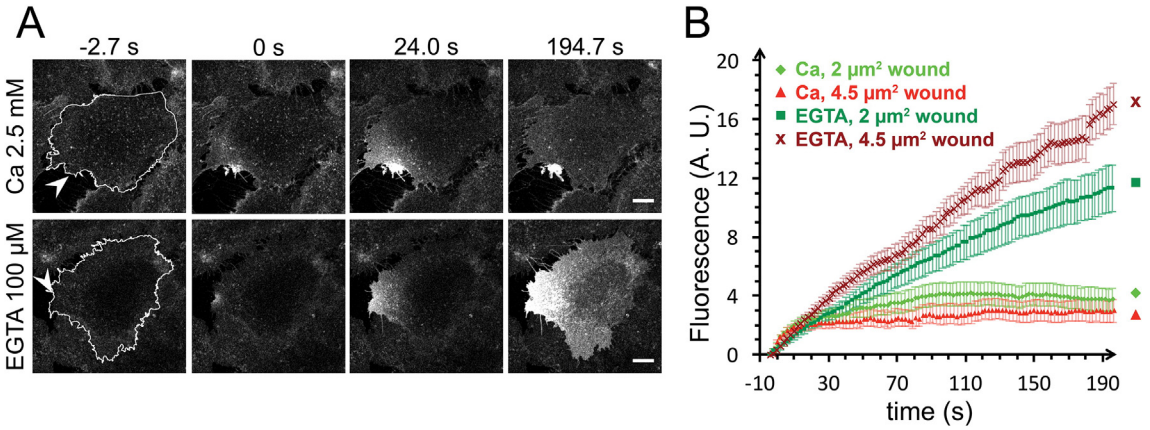

Fig. 1. HUVEC reseal plasma membrane injuries in the presence of Ca^{2+}. A) HUVEC were injured at t = 0 by irradiating a 2 µm^2 circular region of interest (ROI) with 820 nm laser light in the presence of 5 µg/ml FM4-64 in Na-Tyrode's buffer supplemented with either 2.5 mM Ca^{2+} or 100 µM EGTA. Scale bars = 10 µm. B) FM4-64 dye influx into the wounded cell was quantified over time for wounding ROIs of either 2 µm^2 or 4.5 µm^2. The increase in fluorescence intensity is depicted, normalized to both fluorescence intensity before wounding and fluorescence intensity of an uninjured neighbouring cell. n = 10–15 cells per condition from 2 to 3 independent experiments. Error bars are SEM.

phospholipid binding proteins, namely AnxA1 and A2 [14]. This is of special interest because both annexins are also dysregulated in dysferlinopathic patients [15] and because several annexins are recruited to sites of plasma membrane injury in a Ca^{2+}-regulated manner [13,16–19]. Moreover, while a functional contribution of AnxA2 to the membrane repair process has not been elucidated, AnxA1 has been shown to be required for efficient membrane resealing [16]. AnxA5 and AnxA6 are two other annexins that also participate in membrane resealing in certain cell types [13,20]. Mechanistically, AnxA5 has been shown to erect a semi-crystalline membrane scaffold at the site of

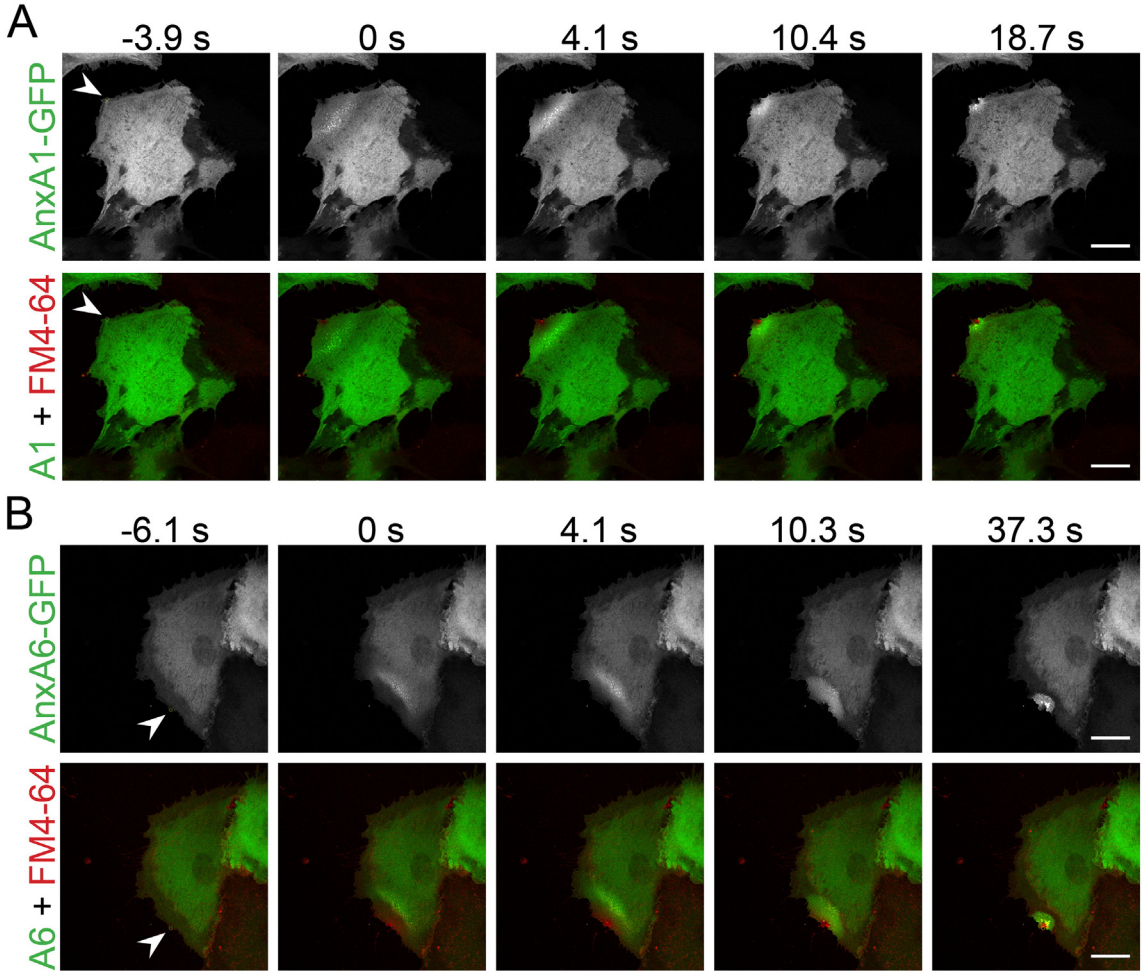

Fig. 2. AnxA1-GFP and AnxA6-GFP are recruited to sites of endothelial plasma membrane injury. A) A ROI of 2 µm^2 of a HUVEC transfected with AnxA1-GFP was irradiated with 820 nm laser light 24 h post transfection. Success of plasma membrane rupture was assessed by FM4-64 imaging. B) Wounding of a HUVEC overexpressing AnxA6-GFP was performed as above. Scale bars = 20 µm.

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