The shear stress of it all: the cell membrane and mechanochemical transduction

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As the inner lining of the vessel wall, vascular endothelial cells are poised to act as a signal transduction interface between haemodynamic forces and the underlying vascular smooth-muscle cells. Detailed analyses of fluid mechanics in atherosclerosis-susceptible regions of the vasculature reveal a strong correlation between endothelial cell dysfunction and areas of low mean shear stress and oscillatory flow with flow recirculation. Conversely, steady shear stress stimulates cellular responses that are essential for endothelial cell function and are atheroprotective. The molecular basis of shear-induced mechanochemical signal transduction and the endothelium’s ability to discriminate between flow profiles remains largely unclear. Given that fluid shear stress does not involve a traditional receptor/ligand interaction, identification of the molecule(s) responsible for sensing fluid flow and mechanical force discrimination has been difficult. This review will provide an overview of the haemodynamic forces experienced by the vascular endothelium and its role in localizing atherosclerotic lesions within specific regions of the vasculature. Also reviewed are several recent lines of evidence suggesting that both changes in membrane microviscosity linked to heterotrimeric G proteins, and the transmission of tension across the cell membrane to the cell–cell junction where known shear-sensitive proteins are localized, may serve as the primary force-sensing elements of the cell.

Keywords: mechanochemical signal transduction; shear stress; G proteins; membrane fluidity; atherogenesis

1. INTRODUCTION

Over the last decade, great strides have been made in the field of bioengineering with regards to the development of biologically cultured vascular grafts. Key to the development of the ideal cultured vascular implant is the need to bestow the graft with a functional endothelial monolayer. The endothelial monolayer serves as a dynamic interface between the circulating blood elements and the interstitial tissues. Disruption of the monolayer’s integrity and permeability characteristics may permit the localized influx of circulating low-density lipoproteins and other proinflammatory macromolecules into the artery wall (Hunt et al. 1996), which may ultimately contribute to the failure of the vascular graft. It has long been recognized that the endothelial monolayer has the ability to sense and discriminate between haemodynamic forces. The response of the endothelial cells to these haemodynamic forces directly dictates the integrity and permeability, as well as the function, of the monolayer as a whole.

This review will provide an overview of the haemodynamic forces experienced by the vascular endothelium. We will also address the role of the cell membrane and the cell–cell junction as a shear-sensitive mechanoreceptor. Finally, we propose a comprehensive hypothesis based upon the experimental findings from our laboratory and others that describes a possible mechanism of mechanochemical signal transduction in endothelial cells.

2. AN OVERVIEW OF HAEMODYNAMIC FORCES

Mechanical forces are important modulators of endothelial cells. The endothelium responds rapidly and sensitively to the mechanical conditions created by blood flow (Frangos et al. 1985; Rubanyi et al. 1986). As blood flows through a vessel, it exerts a physical force on the vessel wall. This force generates stress which can be resolved into two principal vectors. (i) The stress parallel to the vessel wall is defined as shear stress. This represents the frictional force that blood flow exerts on the endothelial surface of the vessel wall. (ii) The stress perpendicular to the vessel wall is defined as tensile stress. This represents the frictional force that blood flow exerts on the endothelial surface of the vessel wall.

In vitro steady shear-stress flow patterns are typically generated using a continuous flow loop or a syringe pump programmed to deliver a specific and constant flow rate across a cultured endothelial monolayer in a geometrically uniform flow chamber. Typically, once flow is fully established in an experimental preparation, the endothelial monolayer is exposed to temporally and spatially uniform fluid shear stress. Such laminar unidirectional flow is known as mean positive shear stress (MPSS). Temporal shear-stress gradients are defined as the increase or decrease of shear stress over a small period of time at the same location. The more rapid the change in flow, the greater the temporal gradient. Spatial shear-stress gradients are defined as...
the difference of shear stress between two close points of a cell at the same point in time. Both temporal and spatial gradients in shear stress can be generated in vitro and are dependent upon the initial onset of flow and the geometry of the flow chamber, respectively (Haidekker et al. 2001 and are dependent upon the initial onset of flow and the spatial gradients in shear stress can be generated in vitro and are dependent upon the initial onset of flow and the geometry of the flow chamber, respectively (Haidekker et al. 2001; White et al. 2001). In vitro temporal gradients can be virtually eliminated if the onset of flow is slowly ramped up over time (more than 30 s) rather than there being a sudden onset of flow (Haidekker et al. 2001; White et al. 2001). As such, individual components of fluid shear stress can be studied in isolation or combination using a variety of flow profiles (figure 1). Conversely, some in vitro preparations may unintentionally generate a significant temporal component with the sudden onset of flow. Furthermore, the interpretation of many in vitro studies may be complicated by undefined flow profiles or lack of adherence to a uniform nomenclature to describe experimental flow profiles. Apparent conflicting findings reported between studies may often be reconciled when the details of the various flow regimes are closely scrutinized.

The in vivo definition of MPSS should be distinguished from the steady shear-stress flow patterns that are often used in vitro experimental preparations. Arterial blood flow in vivo is pulsatile. Given the pulsatile nature of the cardiac cycle, the absolute shear stress varies throughout the cardiac cycle. In regions where stable flow is unidirectional with no recirculation of flow, the time-averaged fluctuations in shear stress are positive (where positive flow indicates flow in the forward direction and negative indicates flow in the reverse direction). MPSS greater than 6 dyne cm⁻² in magnitude predominates throughout much of the major arterial vasculature. Therefore, in vivo MPSS flow patterns are comprised distinct superpositioned steady and temporal components. We have previously demonstrated that endothelial cells can discriminate between the superposition components of flow and respond differently via distinct mechanochemical transduction pathways (Frangos et al. 1996).

Departures from unidirectional flow and MPSS occur mainly around branch points and distal to stenoses. In idealized in vitro geometries, relatively predictable secondary flow patterns of separation, reattachment and recirculation tend to form (recirculating flow; figure 2a). In vivo anatomical perturbations may result in some departure from these idealized flow patterns, but generalized secondary recirculation patterns remain. Within these regions of recirculating flow, haemodynamic forces interacting with an active vascular endothelium have long been implicated in the non-random localization of atherosclerotic lesions. In the region preceding the carotid bifurcation and along the inner wall of the flow-divider side, flow patterns remain relatively undisturbed and positive. Flow at these sites is primarily unidirectional and mean wall shear stress is high (figure 2b). The occurrence of plaque formation within these regions is correspondingly very low (Swindland 1983). Lesions are distributed mainly along the outer walls of the bifurcation within the region of recirculating flow where mean wall shear stress is relatively low. Mean wall shear stress is low within these regions due to a stagnation point (where shear stress is zero) that forms at the point of flow reattachment. During the downstroke of the systole phase in the cardiac cycle, a reversal of flow occurs, which alters the size and spatial migration of the secondary flow patterns (figure 3; Glagov et al. 1988). As the stagnation point migrates back and forth along the outer wall of the bifurcation throughout the cardiac cycle, the time-averaged shear stress in that region is correspondingly low (figure 2c). Within regions of recirculating flow, significant temporal and spatial gradients of shear stress are also generated (Zarins et al. 1983; Ku et al. 1985; Glagov et al. 1988). Spatial gradients are generated primarily within recirculation zones and at bifurcation flow dividers. Given the pulsatile nature of blood flow, in vivo temporal gradients are generated throughout the vasculature to some degree, but they are significantly magnified within recirculation zones immediately adjacent to the interface between the main flow and the

Figure 1. Sample fluid-flow profiles designed to isolate specific elements of fluid shear stress. (a) Ramped transient (solid line) and a single impulse of flow (dotted line). Both flow profiles expose the monolayer to the same level of shear stress with minimal fluid flow, but in the presence (impulse) or absence (ramped transient) of a temporal gradient. (b) Step flow (solid line), ramp flow (grey line) and 1 Hz pulsatile (dotted line). Step flow exposes cells to a single temporal gradient followed by a variable duration of steady flow. Extended exposure to steady flow (less than 30 min) suppresses the impact of the temporal gradient. Ramp flow delivers steady fluid flow to the monolayer, but contains no temporal component. Pulsatile flow delivers an extended period of temporal gradients, but no steady flow.
recirculation zone. Within recirculation zones, both maximal spatial and temporal gradients overlap each other (Haidekker et al. 2001; White et al. 2001). Although earlier reports suggest that spatial gradients induce a mitogenic response in the endothelial monolayer (DePaola et al. 1992; Davies 1995), more recent and carefully designed studies have demonstrated that spatial gradients in shear stress devoid of a temporal component do not affect endothelial proliferation (Haidekker et al. 2001; White et al. 2001, 2005).

The complex flow profiles within recirculation zones should not be confused with turbulent flow. Turbulence implies random movement of elements in the flow field. Extreme or abrupt changes in geometry distal to severe stenoses, around projecting edges, or about other obstacles in the flow stream may cause focal turbulence in the bulk of the vessel. Although a viscous sublayer where the flow becomes dominated by viscosity may exist on the boundary of flow along the vessel wall, flow fluctuations from the turbulent fluctuations in the bulk flow cannot occur.

![Image](http://rstb.royalsocietypublishing.org/)

**Figure 2.** (a) Representation of flow features at the carotid bifurcation. Change in wall shear stress throughout the course of the cardiac cycle at two locations within the carotid bifurcation. (b) Shear stress along the inner flow-divider wall of the bifurcation. (c) Shear stress within the zone of recirculating flow along the outer lateral wall of the bifurcation. Reproduced with permission from Ku et al. (1985).

![Image](http://rstb.royalsocietypublishing.org/)

**Figure 3.** The spatial migration of the recirculation zone during the sudden onset of flow. Flow lines are given for fluid flow through a sudden-expansion flow chamber. The recirculation eddy grows and the reattachment point (arrow) moves downstream. Cells at any given location beneath the developing eddy (dashed line) experience strong changes of shear stress (i.e. temporal gradients) as the recirculation zone passes over them.
flow can still be transferred to the boundary. Only in a time-averaged sense can the viscous subregion be considered laminar-like flow. Nevertheless the gradient of this flow profile will be much higher than in a fully developed laminar flow. A turbulent flow pattern will also be unsteady and so there is likely to be a significant difference in the shear stresses an endothelial cell experiences in regions of turbulent flow when compared to regions of MPSS. Given that turbulent flow accounts for a very small fraction of the total flow throughout the vasculature, it is unclear if turbulent flow plays a significant pathophysiological role in plaque development. Arterial regions immediately distal to severe stenoses, where turbulence can occur, have not been shown prone to plaque localization (Bomberger et al. 1980; Khalifa & Giddens 1981).

3. THE ROLE OF HAEMODYNAMIC FORCES ON THE ENDOTHELIUM

The nature and magnitude of shear stress at a given location within the vasculature plays an important role in the long-term health of the blood vessel. Although some earlier studies indicated that acute levels of high shear stress may lead to endothelial surface degeneration and erosion (Fry 1968; Langille 1984), most lines of evidence are consistent with the view that a chronic exposure of endothelial cells to high levels of shear stress with little temporal fluctuation promotes an atheroprotective phenotype (reviewed in Davies 1995; Traub & Berk 1998). MPSS promotes release of atheroprotective factors from endothelial cells that inhibit coagulation, migration of leukocytes and smooth-muscle proliferation.

MPSS may also be critical for endothelial cell survival. A number of investigators have demonstrated that MPSS is required for optimal regeneration of an injured endothelium (Levesque et al. 1990; Vyalov et al. 1996; Albuquerque et al. 2000). While MPSS may be necessary for endothelial cell integrity, it also seems to inhibit endothelial proliferation. Endothelial turnover in regions of MPSS, or when cultured under flow, is extremely low (Wright 1972; Levesque et al. 1990). The morphology of endothelial cells within regions of recirculating flow is also significantly different from cells located within regions of MPSS (Flaherty et al. 1972; Dewey et al. 1981). Cells in these low mean shear-stress regions are not aligned and are characterized by a rounded shape, an increased proliferation rate and increased permeability (Langille & Adamson 1981; Levesque et al. 1986; Okano & Yoshida 1992, 1993). The lack of streamlining in the macroscopic topography of the luminal endothelial surface in rounded and non-aligned cells may expose the cells to high spatial shear-stress gradients (Davies 1995). Increased endothelial turnover in regions of recirculating flow has long been implicated in the process of atherogenesis (Wright 1972; Chiu et al. 1998). A number of studies have demonstrated enhanced macromolecular permeability of aortic endothelial cells during mitosis (Caplan & Schwartz 1973; Lin et al. 1988). When a cell within a monolayer undergoes replication, the integrity of the monolayer at that location is temporarily disrupted. The low shear stress surrounding the stagnation point of the flow reattachment site may allow prolonged residence times for circulating proinflammatory cells to adhere to the endothelial monolayer of the vessel (Glagov et al. 1988).

Not surprisingly, endothelial cell geometry and surface topography are also influenced by the magnitude and localization of haemodynamic forces acting at the endothelial surface. Endothelial cells located within regions of positive shear stress are aligned with their longitudinal axis parallel to the direction of blood flow (Flaherty et al. 1972; Dewey et al. 1981). This orientation streamlines the endothelial cell and effectively decreases drag resistance (Barbee et al. 1994, 1995). Thus, it appears MPSS acts as an endothelial cell survival factor rather than a growth factor (Traub & Berk 1998).

4. THE ROLE OF THE CELL–CELL JUNCTION IN MECHANO CHEMICAL SIGNAL TRANSDUCTION

The molecular basis of shear-induced mechanochemical signal transduction and the endothelium’s ability to discriminate between flow profiles remains largely unclear. Given that fluid shear stress does not involve a traditional receptor/ligand interaction, identification of the molecule(s) responsible for sensing fluid flow and mechanical force discrimination has been difficult. It has been suggested that the lipid bilayer itself may act as a flow-sensing receptor (Katoh et al. 1995; Gudi et al. 1998a,b). Fluid shear stress is a frictional force that acts on the apical surface of the endothelial monolayer. When a mechanical force is applied to a non-ridged surface such as a plasma membrane, force is transmitted throughout the cell to the regions of the cell that most resist deformation. In a confluent endothelial monolayer, the region of greatest mechanoforce transduction may be the cell–cell junction. Although endothelial cells tend to align themselves with the direction of flow to minimize drag resistance, fluid shear stress imparts a significant level of tension across the whole of the cells apical surface area. The directional vector of the tension is opposite the direction of the fluid flow. As such, a gradient of tension is generated across the apical surface of the cell membrane and is greatest at the upstream cell–cell junction (Fung & Liu 1993; Dussere et al. 2004). This phenomenon can be termed flow-induced propagation of tension (figure 4). It follows that temporal gradients in flow would lead to the highest tension rates at the cell–cell junction as opposed to the rates of tension generated from MPSS. Differences in rates of tension at the cell–cell junction represent a possible mechanism for the endothelium’s ability to discriminate between various flow profiles. As we will discuss later in §5 of this review, it is our hypothesis that large tension rates may also lead to transient membrane destabilization.

When endothelial monolayers are exposed to increasing levels of force from fluid shear stress, cell-cell junction-associated proteins undergo significant dose-dependent reorganization, whereas the integrity of the monolayer remains unaffected (Seebach et al. 2000). A number of membrane-associated proteins are specifically localized to the cell–cell junction. Platelet endothelial cell adhesion molecule-1 (PECAM-1) is a
cell adhesion molecule localized to the interendothelial cell–cell adhesion site. PECAM-1 (also known as CD31 or endoCAM) is the cell–cell adhesion molecule most abundantly expressed in endothelial cells. It is also expressed by platelets, monocytes, neutrophils and a certain subset of T-lymphocytes (Newman et al. 1990). In cultured endothelial cells, PECAM-1 is diffusely distributed in the plasma membrane of solitary cells, but once a cell–cell contact is made, it accumulates at the contact site. In endothelial cells forming a confluent monolayer, it is exclusively localized along the cell–cell border (Albelda et al. 1990). When confluent endothelial cells are exposed to physiological levels of fluid shear stress, PECAM-1 has been shown to be tyrosine-phosphorylated (Harada et al. 1995). Other studies strongly suggest that mechanically induced tyrosine phosphorylation of PECAM-1 is not a downstream event of Ca\textsuperscript{2+} mobilization, K\textsuperscript{+} channel activation, stretch-activated cation channel activity or PKC activation (Ando et al. 1988; Olsen et al. 1988). Given the localization of PECAM-1 to regions of high-mechanoforce transduction, shear-induced PECAM-1 phosphorylation may result from force-induced deformational changes in the molecule.

The rapid activation of heterotrimeric G proteins is known as an early flow-mediated response in endothelial cells (Gudi et al. 1996), and may also play a role in the ability of the endothelium to discriminate between flow profiles. In confluent endothelial monolayers, the sudden temporal onset of flow induces a burst of NO production. This process is both calcium and G protein dependent. In contrast, the prolonged steady shear stress that follows induces a sustained release of NO, at a rate of 10% of the initial peak of NO production, and is both calcium and G protein independent (Kuchan et al. 1994). This suggests that shear-induced stimulation of endothelial cells is derived from the superposition of two independent mechanical stimuli (steady shear and temporal changes in shear stress), which are in turn transduced by two different mechanochemical pathways. Furthermore, membrane-linked force transduction appears to be mediated, at least in part, by the G\textsubscript{q} heterotrimeric G protein.

Studies in our laboratory have demonstrated the localized molecular associations and signalling events of G\textsubscript{q} activation at the cell–cell junctions in association with PECAM-1 (Otte et al. submitted). G\textsubscript{q} is localized to the endothelial cell–cell junction and its shear-regulated link to PECAM-1. In wild-type mice, G\textsubscript{q} forms a distinct ring around the periphery of the cell. This staining pattern is consistent with PECAM localization, which in the same vessel is also localized to the periphery of the cell. In PECAM-1 knockout mice, G\textsubscript{q} is no longer localized to the periphery of the cell and is distributed around the nucleus. When either G\textsubscript{q} or PECAM-1 was immunoprecipitated from human umbilical-vein endothelial cells (HUVEC) lysates, both proteins were found to co-precipitate. This strongly suggests that G\textsubscript{q} is physically linked to PECAM-1 in a complex at the cell–cell junction. The co-localization of G\textsubscript{q} and PECAM-1 was found to be sensitive to temporal gradients in shear stress. When the effects of the temporal gradient were eliminated from the chamber by slowly ramping the onset of flow, it was found that flow alone could not stimulate G\textsubscript{q}–PECAM-1 disassociation. However, when impulse flow was applied to the HUVEC monolayer, temporal gradients were found to be potent mediators of PECAM-1 and G\textsubscript{q} disassociation.

PECAM-1 may play an additional role within the cell–cell junction in the endothelium’s ability to discriminate between temporal gradients in shear stress and steady fluid-flow-mediated NO release and dilution (Bagi et al. 2005). Mice with a genetic ablation of PECAM-1 display impaired regulation of arteriolar dilatation in response to sudden changes in fluid flow (temporal gradients in shear stress). Diminished sensitivity to temporal gradients in shear stress was characterized by reduced arteriolar dilatation in PECAM-1 knockout when compared to wild-type mice. Moreover, in arterioles of PECAM-1 knockout mice, NO-mediated vasodilatation in response to temporal gradients in shear was absent, whereas the shear-independent release of NO (via the Ca\textsuperscript{2+} ionophore A23187) was similar to that in wild-type mice. Collectively, these findings strongly suggested that arterioles of PECAM-1 knockout mice are less sensitive to temporal gradients in shear stress than control vessels and is consistent with previous studies which found that sudden increases in wall shear stress is the main stimulus for NO release (Frangos et al. 1996; Dusserre et al. 2004).

PECAM-1 may indeed represent a link between the primary force-sensing elements of the cell and downstream mechanochemical transduction pathway. The cytoskeleton has long been suggested to play a role in the downstream mechanochemical transduction pathway (Davies 1995), but its possible role as a primary sensor of flow has never been convincingly demonstrated. Pharmacological disruption of the actin cytoskeleton does not significantly affect shear-induced endothelial response (Knudsen & Frangos 1997). Paradoxically, in studies with knockout mice, the deletion of vimentin, desmin and dystrophin (specialized cytoskeletal components) significantly reduced (but did not eliminate) shear-induced endothelium-dependent vasodilation (Loufrani et al. 2001, 2002). Taken
Mechanochemical signal transduction is the process that enables cells to perceive and respond to stimuli such as strain, pressure and fluid shear stress. We have proposed (Davies 1995), as the direct stimulation of transmembrane proteins exposed on the luminal surface, activation of ion channels which alter membrane polarization and/or intracellular Ca$^{2+}$, or transduction of stress along cytoskeletal elements to other regions of the cell. An earlier study suggested a role for membrane permeability. It was demonstrated that shear stress causes increased membrane permeability to the amphipathic dye merocyanine 540, which suggests a fluid-induced increase in membrane fluidity (Berthiaume & Frangos 1994). Presently, several newer lines of investigation strongly suggest that the apical surface of the endothelial cell membrane and its microviscosity changes when exposed to varying profiles in shear stress may act as a primary mechanoreceptor of fluid shear stress.

Membrane microviscosity is a physical property of the cell, which describes the movement of molecules with the phospholipids layer. In general, membrane microviscosity depends on the chemical composition of the bilayer and is shown to have optimum values for the proper function of various membrane-bound enzymes and receptors (Kung & Reed 1986; Kapitulnik et al. 1987; Dunham et al. 1996). It can be quantified using a concept of membrane free volume $v_f$ (Cohen & Turnbull 1959). It characterizes the difference in molecular volumes of the membrane at a given temperature with respect to molecular volume at absolute zero $v_f = v - v_0$. The membrane microviscosity $\eta$ can be directly related to the membrane free volume according to the following expression (Doolittle 1951):

$$\eta = A e^{B(v_f/v_i)} ,$$

where $A$ and $B$ are constants. In the case of orientational motion, membrane free volume defines relative volume available for reorientational motion of the probe molecule. Molecular rotors exhibit viscosity-dependent fluorescence quantum yield due to a non-radiative deactivation channel. The non-radiative deactivation of molecular rotors is mediated by isomerization (intramolecular rotation of a part of the molecule) and therefore higher viscosity tends to inhibit this rotation, leading to higher fluorescence quantum yield. The changes in the quantum yield in this case are mostly determined by the non-radiative rate deactivation rate, $k_{\text{nonrad}}$ which can be related to the free volume, $v_f$:

$$k_{\text{nonrad}} = k_0 e^{-\beta(v_f/v_i)},$$

where $k_0$ and $\beta$ are constants. By measuring quantum yield dependence of a molecular rotor incorporated into membrane, one can in principle determine the membrane free-volume changes. We have performed fluid shear-stress experiments with a lipid molecular rotor FCVJ 9-(2-carboxy-farnesylester-2-cyanovinyl)-julolidine. Figure 5 shows fluorescence intensity of FCVJ incorporated into membranes of confluent layers of HUVEC cells exposed to variable fluid shear stress. The results indicate that upon application of a shear stress ramp, FCVJ fluorescence intensity proportionally decreases suggesting that membrane tension is leading to larger membrane free volume (higher fluidity; Haidekker et al. 2000, 2002).

We hypothesize that fluid shear stress causes activation of G proteins in liposomes in complete absence of G protein-coupled receptors (Guidi et al. 1998a,b). According to the Kramers reaction rate theory (Hanggi et al. 1990), reaction rate is inversely proportional to viscosity, thus it can be related to the free volume parameter

$$k_{\text{cat}} = \frac{C}{\eta} e^{-\frac{\Delta H}{RT}} = A e^{-\frac{\Delta H + \gamma(v_f/v_i)RT}{RT}},$$

where $\Delta H$ is the activation energy of the reaction, $\gamma$ is the viscosity coefficient, and $\eta$ is the membrane viscosity.
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6. CONCLUSION
As highlighted in this brief review, many of the biochemical force-transduction pathways have been characterized. However, the primary mechanoreceptor(s) remain unknown. It is our hypothesis that hydrodynamic shear destabilizes the plasma membrane, leading to a decrease in membrane microviscosity, or more precisely, an increase in membrane free volume. Changes in membrane microviscosity directly activate various secondary signal cascades linked to heterotrimeric G protein. Tension generated across the cell membrane by fluid shear stress is transmitted to the cell–cell junction where known shear-sensitive proteins are localized. Furthermore, endothelial cell differentiation between MPSS and temporal gradients in shear stress takes place primarily at the cell–cell junction and is dictated by the rate of tension generated between the two flow profiles. This model of mechanochemical transduction in the vasculature provides a comprehensive basis for mechasignalling in endothelial cells and a foundation for therapeutic strategies for treatment of cardiovascular disease.

This work was supported by a grant from the National Institutes of Health HL40696.

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