Vascular leakage is commonly observed in patients suffering from many different pathological conditions. It is often complicating these pathologies, but may also be the underlying cause. As described in **chapter 1**, vascular leakage is caused by an increase in endothelial permeability. Endothelial cells, by tightly adhering to their neighbors, form a physical barrier that normally prevents vascular leakage. Disruption of these adhesion complexes, in particular the adherens junctions (AJs), causes interendothelial gap formation. AJ disruption is accompanied by the liberation of junctional proteins like β-catenin and p120ctn from the cell membrane. Interestingly, during vasculogenesis and under pathological conditions nuclear localization of β-catenin is associated with enhanced gene-expression that might alter the vasculature.

Several vasoactive agents like thrombin and VEGF can induce endothelial permeability *in vivo*. It is known that this involves activation of the small GTPase RhoA and its downstream target Rho kinase, and subsequent stress fiber (SF) formation and cell contraction. Possibly, RhoA/Rho kinase are also directly involved in the disruption of the junctional complexes, which via β-catenin liberation may result in gene expression. This thesis therefore focused on how RhoA and Rho kinase regulate the endothelial barrier and whether permeability-mediated β-catenin liberation induces gene expression.

In **chapter 2**, the proteins that control the activities of the Rho GTPases RhoA, Rac1 and Cdc42 during endothelial barrier regulation are reviewed. The importance of the spatio-temporal regulation of Rho GTPases by the currently known GEFs, GAPs and GDIs during thrombin- and VEGF-induced endothelial permeability is emphasized. Understanding the spatio-temporal regulation of RhoA in endothelial barrier maintenance and disruption is a central issue in chapter 5, where we investigate the subcellular localization of RhoA activity in endothelial monolayers.

Many vasoactive agents, including thrombin, induce RhoA/Rho kinase-mediated cell contraction leading to inter-endothelial gap formation and endothelial permeability. In **chapter 3** we demonstrate in non-stimulated microvessels, that intrinsic Rho kinase activity plays a role at the plasma membrane. In combination with experiments showing that basal Rho kinase activity prevents endothelial permeability and the loss of VE-cadherin at the plasma membrane, we propose that Rho kinase activity by recycling of VE-cadherin to the plasma membrane is required for AJ formation. In this chapter we thus provide proof for a dual role of Rho kinase in the regulation of the endothelial barrier.

Meanwhile, evidence was accumulating that suggested a distinct role for the closely-related Rho kinase isoforms ROCK-I and ROCK-II. In **chapter 4,** we therefore investigate the role of ROCK-I and ROCK-II in thrombin-mediated permeability. By using either isoform specific and non-specific pharmacological inhibitors or siRNA against the specific Rho kinase isoforms we obtained *in vitro* data that demonstrates that ROCK-II is mainly involved in thrombin-induced permeability, which occurs independent of SF formation. The data further suggests a role for ROCK-I in ruffle formation and VE-cadherin recycling to the plasma membrane. Strikingly, inhibition of both ROCK-I and ROCK-II are required for the inhibition of thrombin-mediated SF formation. This chapter thus shows that ROCK-I and ROCK-II have a differential role in the regulation of the endothelial barrier. The *in vivo* relevance was verified utilizing specific siRNA against ROCK-I and/or ROCK-II in mice.

Because we hypothesized that knowledge about the spatio-temporal localization of RhoA activity in endothelial monolayers might give further insight in the regulation of endothelial barrier regulation, the

subcellular localization of RhoA activation before and during thrombin-stimulation was visualized. In order to do this, we first had to obtain a sensor that could indicate RhoA activity in living cells in real-time and set-up a validated system for ECs. We appreciate that several RhoA sensors where kindly provided by Prof.dr. M. Matsuda (Osaka University, Japan). In order to correctly analyze the data obtained with these sensors the Triple-I-software (Denver, CO) belonging to our microscope had to be adapted. In **chapter 5** we can now report that in confluent EC monolayers RhoA activity at the plasma membrane occurs prior to gap closure and at the membrane ruffles. Upon thrombin stimulation RhoA is transiently activated at small fin-like ruffles. Additionally, we provide evidence for the activation of RhoA at SFs where it stays activate during fiber remodeling/contraction. Finally, prior to cell separation RhoA activity was located at the finger-like bridges around the interendothelial gaps. We propose that the four subcellular localizations of RhoA activity described in this chapter are linked to processes that regulate endothelial permeability.

In **chapter 6** we show that thrombin-mediated Rho/Rho kinase activity is required for the disassembly of junctional complexes and thus the formation of interendothelial gaps. Thrombin mediates the relocalization of β-catenin and p120ctn from the membrane to the cytosol and the nucleus. Moreover, thrombin induces the inhibitory phosphorylation of GSK3β thereby preventing the ubiquitination and degradation of liberated β-catenin thus stimulating its nuclear translocation. Using a β-catenin/TCF reporter construct it was demonstrated that thrombin-mediated nuclear localization of β-catenin enhances gene expression. Several β-catenin inducible pro-angiogenic genes are indeed enhanced, which suggests that during prolonged endothelial permeability β-catenin may drive vascular remodeling.

The implications of our results obtained in the studies of this thesis are discussed in **chapter 7**, where they are placed in the context of the rapidly developing field of vascular permeability.