***Review Paper:***

# Specific Endothelial Promoters for Targeted Gene Expression and Gene Therapy

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## *Abstract*

*Tissue-specific promoters play a crucial role in both fundamental research and practical applications. They allow targeted gene expression only in specific cell types, minimizing off-target effects on neighboring cells. In the context of gene therapy for vascular diseases, the use of tissue-specific promoters becomes particularly important to minimize non-targeted transgene expression and potential side effects. Endothelial cells, which line blood vessels and regulate the exchange of substances between blood and organs, are potential targets for gene therapy in certain vascular diseases.*

*Several endothelial-specific promoters have been proposed and characterized to date, including Tie1 and Tie2, PECAM-1, ICAM-1, E- and P-selectins, Flt1 and flk-1, endoglin, eNOS, claudin 5, VE-Cadherin, preproendothelin-1 and von Willebrand factor. In this review, we discuss the characteristics and applications of these endothelial-specific promoters. We highlight their advantages and limitations and explore their potential in gene therapy.*

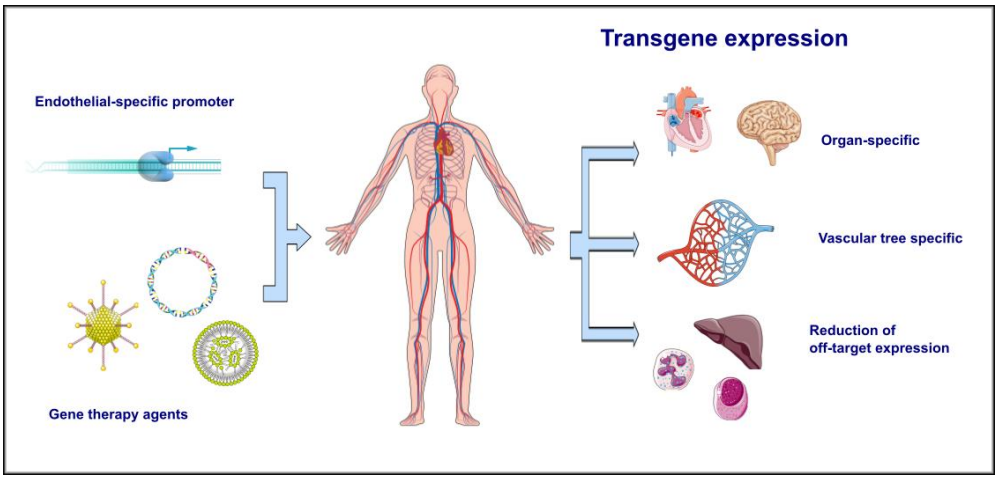
**Keywords:** Specific promoters, targeted expression, endothelium, gene therapy of vascular diseases

## Introduction

Tissue-specific promoters are of great importance in both fundamental research and practical applications. Their use allows targeted gene expression only in specific cell types, without affecting the physiology of neighboring cells. This enables the study of gene effects in specific tissues and minimizes the risks of non-targeted expression of gene therapy products. The use of tissue-specific promoters also allows to study the effects of gene deletion, for example, by induced deletion using Cre recombinase controlled by a specific promoter[6]. The expression of suicide genes in specific cell types allows to investigate the cell death effects on the organism in general or, for example, to reduce tumor growth[23].

However, unfortunately, many promoters are active in multiple cell types which limit their use and complicate the interpretation of the research results. Therefore, experimental output will largely depend on the correct choice of a tissue-specific promoter.

Endothelial cells are cells that line blood vessels and regulate the exchange of substances between blood and organs. Targeted gene expression in endothelial cells has significant clinical implications. For example, in ischemic heart disease, many patients require surgical revascularization with autologous vein grafts. The need to prevent vein graft failure has led to great interest in gene therapy approaches to address this problem[51]. Potential gene therapy targets for atherosclerosis and varicose veins have also been proposed[3153]. Additionally, in the context of tumors gene therapy, blocking angiogenesis through targeted expression of suicide genes in dividing endothelial cells is a promising strategy[49].




**Graphical Abstract**

Also recently, the role of blood vessels as targets for genetic therapy of neurodegenerative diseases has been discussed [22]. There is a report of successful application of endothelial-targeted genetic therapy for Sandhoff syndrome in mice. This inherited neurodegenerative disease is characterized by a deficiency of the lysosomal enzyme β-hexosaminidase, which leads to the accumulation of gangliosides in neurons and triggers neurodegeneration. Targeted expression of β-hexosaminidase subunits A and B in brain endothelial cells reduced the symptoms of this disease [14].

Thus, the choice of a promoter for endothelial expression is of great importance both in theoretical studies and in the design of a vector for gene therapy. The aim of this review is to summarize information about promoters used as endothelium-specific, to discuss their advantages and limitations and to explore their potential for gene therapy.

## Endothelial-specific promoters

Promoters can be recognized by different classes of RNA polymerases. Protein-coding genes are mainly regulated by promoters recognized by RNA polymerase II, while non-coding RNAs are generally regulated by promoters for RNA polymerases I and III[33]. It is worth noting that information about tissue specificity of promoters for RNA polymerases I and III is limited. We will only consider those promoters that are recognized by RNA polymerase II.

Native promoters can originate from genes encoding structural proteins (intercellular contact proteins, cytoskeleton proteins etc.) or regulatory proteins (receptors, signaling peptides, enzymes etc.). It is worth noting that the same promoter may not always result in the same level of expression and tissue localization of a transgene in comparison with a native gene regulated by that promoter. For example, although a particular promoter may drive expression of the native gene in all endothelial cells, the expression of the transgene may be limited to specific organs or regions of the vascular tree[4,10,11,19,21,29,41]. This can make it challenging to select a promoter when uniform expression is desired in all vessels simultaneously. However, this feature can be used for localized expression of the transgene. For instance, some promoters may not drive transgene expression in the liver, which theoretically reduces non-specific toxicity from the transgene and makes them attractive for designing gene therapy agents[40,44].

Promoters can originate from various organisms. The study of human promoters in model organisms is of great interest for medical purposes, with the aim of translating these findings to humans. However, it is challenging because the same promoter may function differently in different organisms. For example, the human ICAM-2 promoter drove high levels of expression in mouse endothelium but not in pigs[10].

When selecting an endothelial promoter, it is also important to consider that endothelial cells are heterogeneous. Transcriptomic analysis has shown that endothelial cells vary depending on the blood vessel type (large arteries, arterioles, capillaries, venules and veins) and the organ they are found in[16,28,45]. Some endothelial cells are so distinct from others that they have their own type-specific promoters. For example, specific promoters for blood-brain barrier endothelium can be used for targeted expression in the central nervous system (CNS)[35].

Promoters can function differently depending on the type of blood vessel. Some primarily drive expression in small vessels while others are preferentially active in larger vessels[10]. Promoters also differ in their activity in dividing and mature endothelial cells. This allows the use of promoters that specifically target dividing cells, for example, to block angiogenesis [49 50].

Many endothelial promoters also exhibit activity in hematopoietic cells (macrophages, lymphocytes, megakaryocytes and other CD31+ cells)[11,20,29,32,40]. Transgenic animal studies have shown that non-targeted expression of a transgene in hematopoietic cells can disrupt hematopoiesis and can lead to diseases[29]. Therefore, to minimize potential side effects of gene therapy on hematopoiesis, it is crucial to use a promoter that is inactive in hematopoietic cells.

The promoter regions upstream of several endothelial cell-specific genes have been characterized. Using these promoters plasmids, various viral vectors and transgenic animals have been obtained. Most of these promoters contain binding sites for both specific and nonspecific transcription factors. There is no common structure or arrangement of the transcription binding DNA elements. Thus, it is impossible to identify any universal structure characteristic of endothelial-specific promoters [13].

**1. Tie2:** Promotor of receptor tyrosine kinase from mouse. Size ~ 2.6 kb. It is widely known for its use in creating a commercially available line of transgenic mice with Cre recombinase expression in endothelial cells. The use of these animals allows to study the conditional knockouts or knockins effects of various genes on different stages of development[30]. The 1.2-kb 5' flanking region of the Tie2 promoter directs gene expression specifically into a subset of endothelial cells in transgenic mouse embryos. However, this activity is restricted to early embryonic stages and is not detectable in adult mice.

By combining the Tie2 promoter (2 kb) with an intron fragment containing the first exon enhancer (1.6 kb), it is possible to target reporter gene expression specifically and uniformly to virtually all vascular endothelial cells throughout embryogenesis and adulthood[48]. However, it has been shown that Tie2 promoter activity is not homogeneous throughout the vascular tree and is asymmetrically distributed, being much stronger in arteries and arterioles than on the venular side. The capillaries exhibit a mosaic

pattern of Tie2 promoter activity [4]. Although this promoter is considered highly specific for endothelium, it also provides transgene expression in hematopoietic cells and in mesenchymal cells of some vessels[30,32,48].

**2. ICAM-2:** Promoter of intercellular adhesion molecule 2 from human. Size ~ 0.3 kb. The ICAM-2 promoter is TATA-less, containing a Sp1 site, two GAGA sites and an 8-bp palindromic sequence[12]. The advantage of this promoter is its small size, which allows it to be used in vectors with small coding capacity such as adeno-associated viral vectors[12]. The ICAM-2 promoter has been used to generate transgenic animals that demonstrate endothelial expression of a transgene.

Strong and uniform expression was observed on the endothelial cells of all blood vessels in the heart, kidney, lung, liver and pancreas. Little or no expression was seen in other cell types, except for neutrophils and monocytes [11]. Interestingly, transgenic pigs expressing the transgene under the same promoter showed expression that appeared to be restricted to vascular endothelium in the heart and kidney but markedly weaker than in transgenic mice. Thus, in this case, promoter performance in mice and pigs was not equivalent. The weak expression driven by the human ICAM-2 promoter in pigs relative to mice suggests the need for additional regulatory elements to achieve species-specific gene expression in pigs[10].

Synthetic ICAM2 is made based on the ICAM-2 promoter and several transcription factor binding sites. The promoter was selected by high-throughput screening from a set of randomly ligated fragments. When used in lentiviral vectors, the obtained promoter had a high level of expression and selectivity in endothelial cells (tested on Bovine aortic endothelial cells, Rabbit endothelial venous cells and choroid plexus endothelium) [13]. The artificial ICAM-2 promoter demonstrates higher expression and specificity compared to the native promoter. It inherited the small size of the natural promoter, not exceeding 300 nucleotides [13].

**3. Endoglin:** Promoter of endoglin (CD 105) from human. Size ~ 0.9 kb. The promoter contains consensus motifs for TGF-beta-, glucocorticoid-, vitamin D-, estrogen-responsive and hypoxia-sensitive elements[46,47]. In transgenic mice, expression from the endoglin promoter was consistently strong in the small vessels but was absent in larger vessels[10]. Thus, this promoter could potentially be used for targeted expression in arterioles, capillaries and venules.

**4. Flt-1:** Promoter of vascular endothelial growth factor receptor 1 (Flt-1/VEGFR1) from human. Size ~ 1 kb. One TATA box, four GC boxes, nine E26 transformation specific (ETS) motifs and one cAMP response element (CRE) motif were found in the upstream region (489 bp) of exon 1[24]. It has high selectivity and provides a high level of expression in endothelial cells[24]. Using an *ex vivo* human gene therapy model, the Flt-1 promoter showed specific transgene expression in human veins but no detectable expression in infected exposed smooth muscle cells.

Additionally, when adenoviruses were administered systemically to mice, the Flt-1 promoter showed very low-level gene expression in the liver, which is the primary target organ for adenoviral transduction *in vivo* and can cause unintended transgene-related toxicity. This highlights the potential of using the Flt-1 promoter for local and systemic human gene therapy[40]. However, like many other promoters, it is also active in some hematopoietic cells[40]. Furthermore, this promoter has high transgene expression levels in teratocarcinoma lines. These results suggest that the Flt-1 promoter could be useful for targeted gene expression in teratocarcinoma and should be evaluated in other Flt-1-positive tumors[5].

**5. Flk1:** Promoter of vascular endothelial growth factor receptor 2 (Flk/VEGFR2) from human. Size ~ 1 kb. The 5'-flanking sequence is GC rich, contains five Sp1 elements and a single transcription start site, but no TATA consensus sequence[43]. Hypoxia-inducible factor-2 alpha (HIF-2 alpha) and an ETS binding site in tandem within the VEGFR-2 promoter act as a strong enhancer element for this gene[15].

It has been shown that this promoter provides transgene expression primarily in proliferating human dermal microvascular endothelial cells in xenograft experiments [49]. This allows the use of this promoter for targeted expression in areas undergoing active angiogenesis. This opens up possibilities for anti-angiogenic gene therapy for tumors. For example, there are reports of the transcriptionally targeted antiangiogenic adenoviral vector construction which mediates neovascular disruption upon activation of a caspase-based artificial death switch [50].

**6. Claudin 5:** Promoter of tight junction protein from mouse. Size ~ 3 kb. The promoter contains six full and two half sites of degenerated glucocorticoid-response elements (GREs), two NFkappaB, three Sp1, one Sp2, one Ap2, as well as three E-boxes[7]. Claudin 5 is a tight junction protein characteristic of blood-brain barrier endothelial cells. It plays an important role in maintaining the barrier properties of the blood-brain barrier and restricts the flow of fluid between cells[18].

Thus, this promoter ensures expression mainly in blood-brain barrier endothelial cells, making it a useful tool for blood-brain barrier research and CNS-targeted therapy. For practical applications, a "mini-version" of this promoter has been proposed, called "Ple261", compatible with using in AAV vectors [35].

**7. VE-cadherin:** Promoter of VE-cadherin (Cdh5) from human. Size ~ 2.5 kb. The VE-cadherin promoter contains a GT box (-48 to -40) and two Ets binding sites (-93 to -90 and -109 to -106)[17].

In adult transgenic mice, the transgene was specifically and strongly expressed in the lung, heart, ovary, spleen and kidney glomeruli. However, expression was weak or absent in the vasculature of other organs including the brain, thymus, liver and skeletal muscle. The VE-cadherin promoter is responsive to bFGF induction. Transgene expression was also observed in extravascular sites of the central nervous system, suggesting that silencer elements may be located elsewhere in the gene[44]. The Cdh5 promoter was found to be active in lymphatic endothelial cells[3]. However, this promoter is not active in hematopoietic cells which distinguishes it favorably from many other endothelial promoters[29].

**8. Tie1:** Promoter of receptor tyrosine kinase from mouse. Size ~ 0.8 kb. The Tie1 promoter does not have the typical TATA and CAAT boxes, but it does contain GC sequences, a GT repeat and binding sites for AP-2, Ets-1 and PEA3. There is an octamer (ATGCAAAT) site at -312 bp that appears to be responsible for endothelial cell-specific gene expression[25,32]. Expression of the transgene driven by this promoter was observed in most endothelial cells in the embryo as well as in extra-embryonic tissues such as the yolk sac and chorioallantoic placenta of transgenic mice. Expression of the transgene was also detected in 12-20% of adult erythroid, myeloid and lymphoid cells as well as in specific regions of the adult brain[20].

**9. Preproendothelin-1:** Promoter of preproendothelin-1 (ppET, the precursor of endothelin-1). Several variants of the ppET promoter have been proposed:

(a) Promoter from mouse, size 0.2 bp[34]. When recombinant adenoviral vectors with ppET-1 promoter were injected systemically into mice, higher activity of this promoter was observed in the aorta and vascularized tissues such as the heart, kidney, lung and pancreas[52].
(b) Promoter from mouse, 5.9-kb 5' flanking region. This promoter was used to generate transgenic mice [21]. It showed high levels of expression in the endothelial cells of both large and small arteries, lower levels of expression in veins and capillaries and significant expression in arterial smooth muscle cells[21].
(c) Synthetic 'ppETlong' promoter from human, size ~0.5 kb. This promoter is based on the ppET promoter fused with regulatory elements from the CMV promoter[8]. This promoter is sensitive to hypoxia and its activity was increased 1.6-fold under hypoxia-mimic conditions[8].
(d) Synthetic ppET promoter from human designed for lentiviral expression, size ~0.3 kb[26]. Overall, all the proposed promoters based on the ppET-1 promoter exhibit strong and relatively specific expression. The small size of these promoters makes them attractive for use in vectors for gene therapy.

**10. PECAM-1:** The promoter of platelet endothelial cell adhesion molecule 1 (CD31) from human. Size - 1.42 kb. The expression from this promoter in transgenic mice was found to be weak in the liver and non-uniform in the small vessels of the heart, kidney and lung[10].

**11. von Willebrand factor:** Promoter of von Willebrand factor (vWF) from human, size 0.73 kb. The 5' flanking region contains an AT-rich region resembling a TATA element and a GT repetitive sequence[37]. Data on this promoter are contradictory. For example, it was shown that when used in adenoviral vector, expression levels were extremely low *in vitro*[40]. However, when used in the herpes simplex virus, the vWF promoter provided efficient transgene expression *in vitro*[41]. In a study on transgenic mice, the authors demonstrated that the vWF promoter allows the reporter gene expression in a subset of endothelial cells in the heart, skeletal muscle and brain[39].

In another study, also conducted on transgenic mice, it is claimed that the activity was absent in the vascular beds of the heart. The vWF promoter targeted expression of the reporter gene to a subpopulation of endothelial cells in the yolk sac and adult brain. The activity was absent in the vascular beds of the spleen, lung, liver, kidney, testes, aorta, as well as in megakaryocytes[2]. When using an extended version of the vWF promoter containing 2.182 bp of 5' flanking sequence to generate transgenic mice, transgene expression was detected within endothelial cells in the brain, heart and skeletal muscle [1].

**12. P-selectin:** Promoter of P-selectin from human. There is a report on its testing on bovine aortic endothelial cells *in vitro* where a plasmid vector was used for transgene delivery[42]. In another study, transgenic mice were created with a human P-selectin promoter instead of their own murine promoter to investigate species-specific regulation of P-selectin expression. The 1.4-kb proximal region of the murine P-selectin promoter was replaced with the corresponding human sequence. These transgenic mice demonstrated a 2-6 fold higher level of basal P-selectin expression compared to wild-type mice. These data suggest that the promoter mediates functionally significant differences in the expression of human and murine P-selectin *in vivo*[36].

**13. E-selectin:** Promoter of E-selectin, a heavily glycosylated transmembrane protein from human, size ~0.9 kb. This promoter allows the induction of expression in an inflammation-specific manner. In *in vitro* experiments, transgene expression under the control of this promoter is increased in response to the induction of an inflammatory reaction using lipopolysaccharides[54]. A mutant version of this promoter is also proposed for use in lentiviral vectors [27].

**14. Pdgfb:** Promoter of platelet derived growth factor subunit B. For the transgenic mice generation, 90-kb genomic sequence upstream of the mouse Pdgfb gene was used[9]. In transgenic mice, transgene expression was observed in all blood vessels except the liver. Expression was also observed in some hematopoietic cells [29].

**Table 1**
**Endothelial-specific promoters and their properties**

| Promoter | Size | Host | Expression in organs and vascular tree | Examples of application | Activity in other cell types |
|---|---|---|---|---|---|
| Tie2 | 2.6 kb | Mouse | In embryos and adult mice. Strong expression in arteries and arterioles, weaker in veins and venules. Nonuniform expression in capillaries | Generation of transgenic mice | Hematopoietic and mesenchymal cells[4,30,32,48] |
| ICAM-2 | 0.3 kb | Human | In mice: endothelial cells of all blood vessels in the heart, kidney, lung, liver and pancreas; In pigs: weak expression in vascular endothelium in the heart and kidney | Generation of transgenic mice and pigs; compatible with AAV vectors | Neutrophils and monocytes[10-12] |
| | 0.3 kb | Synthetic (Human ICAM-2+CMV) | Strong expression in vitro: tested on Bovine aortic endothelial cells, Rabbit endothelial venous cells and choroid plexus endothelium | Lentiviral vectors; compatible with AAV vectors | Unknown[13] |
| Endoglin | 0.9 kb | Human | Strong in the small vessels but absent in larger vessels | Generation of transgenic mice | Hematopoietic cells[10] |
| Flt-1 | 1 kb | Human | Strong expression in endothelial cells, with the exception of liver | Adenoviral vectors | Hematopoietic and tumor cells[5,40] |
| Flk1 | 1 kb | Human | In proliferating microvascular endothelial cells | Adenoviral vectors | Unknown[49,50] |
| Claudin 5 | 3 kb | Mouse | In blood-brain barrier endothelial cells | AAV vectors | Unknown[35] |
| VE-cadherin | 2.5 kb | Human | Strong expression in the lung, heart, ovary, spleen and kidney glomeruli. Weak expression in other organs. | Generation of transgenic mice | Lymphatic endothelial cells[3,29,44] |
| Tie1 | 0.8 kb | Mouse | Expression in embryos (including yolk sac and chorioallantoic placenta) and adult mice | Generation of transgenic mice | Hematopoietic cells[20] |
| ppET | 0.2 kp | Mouse | Strong expression in aorta, heart, kidney, lung and pancreas | Adenoviral vectors | Vectors[52] |
| | 5.9 kb | Mouse | High levels of expression in the large and small arteries, lower levels of expression in veins and capillaries. | Generation of transgenic mice | Arterial smooth muscle cells[21] |
| | 0.3 kb | Synthetic, based on human promoter | Strong expression in vitro | Lentiviral vectors | Unknown[26] |
| PECAM-1 | 1.42 kb | Human | Expression is weak in the liver and non-uniform in the small vessels of the heart, kidney and lung | Generation of transgenic mice | Unknown[10] |
| vWF | 0.73 kb | Human | Extremely low expression in vitro in HUVEC and human saphenous vein endothelial cells | Adenoviral vectors | Unknown[40] |
| | | | Efficient expression in vitro in HUVEC | Herpes simplex virus vector | Unknown[41] |
| | | | Contradictory reports about expression in different organs | Generation of transgenic mice | Hematopoietic cells[2,39] |

| | | | | | |
|---|---|---|---|---|---|
| | 2.1 kb | Human | Expression in endothelial cells in the brain, heart and skeletal muscle | Generation of transgenic mice | Hematopoietic cells[1] |
| P-selectin | 0.25 kb | Human | Expression in bovine aortic endothelial cells in vitro | Transfection with plasmid vector | Unknown[42] |
| | 1.4 kb | Human | Veins of lungs, heart, liver | Generation of transgenic mice | Hematopoietic cells[36] |
| E-selectin | 0.9 kb | Human | Expression in murine endothelioma cells in vitro | Lentiviral vectors | Unknown[27] |
| | | | Expression in human pulmonary artery endothelial cells in vitro | Transfection with plasmid vector | Unknown[54] |
| Pdgfb | 90-kb sequence upstream of the Pdgfb gene | Mouse | Expression in all vessels except the liver | Generation of transgenic mice | Hematopoietic cells[9.29] |
| eNOS | 1.6 kb | Human | Expression in endothelial cells within the heart, skeletal muscle, brain and aorta | Generation of transgenic mice | Unknown[19] |

**15. eNOS:** Promoter of endothelial NO-synthase (eNOS) from human. Size - 1.6 kb. The eNOS promoter does not contain a TATA box, but it has Sp1, Ets, GATA, NF-1, AP-1, shear-stress response elements and sterol regulatory elements. Therefore, various transcription factors known to regulate endothelial gene expression are capable of regulating eNOS promoter activation[38]. Transgenic mice were generated with a construct containing the 1,600-bp 5′ flanking region of the eNOS promoter. Reporter gene expression was restricted to the endothelium; however, expression was limited to a subpopulation of endothelial cells within the heart, skeletal muscle, brain and aorta, but notably absent in other vascular beds that otherwise express the endogenous gene. Notably, the eNOS promoter was selectively upregulated by conditioned media from cardiac myocytes, skeletal myocytes and astrocytes[19].

The information about the promoters is summarized in table 1.

Thus, despite the wide variety of proposed promoters for specific expression in endothelial cells, each of them has its own drawbacks. They include weak expression levels, expression in non-endothelial cells and for some, a large size that prevents their use in vectors with low coding capacity. Some features of certain endothelial promoters include their differential expression in different organs, different sections of the vascular tree as well as in cells with different physiological statuses (proliferating endothelial cells during angiogenesis or mature cells). These characteristics can be used for selective transgene expression in a desired location facilitating an impact on specific organs and cells and reducing the genetic burden of the introduced transgene on the organism in general.

## Conclusion

In conclusion, it should be noted that despite the fact that some promoters have relatively high specificity, there is currently no 100% specific endothelial promoter proposed. For the possibility of using gene therapy methods and targeted gene expression for the treatment of vascular diseases, further search for selective promoters is necessary. Synthetic promoters are promising as they combine different regulatory elements and are more efficient and specific than native promoters.

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