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Commentary

Platelet von Willebrand factor in inherited and acquired bleeding disorders

Pier Mannuccio Mannucci

Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Institute of Internal Medicine, IRCCS Maggiore Hospital and University of Milan, Milan, Italy

The majority of plasma proteins involved in the mechanisms of hemostasis are loosely adsorbed on the platelet surface. A few of them, however, can also be measured in washed platelets, in which they are contained in the intracellular organelles called α granules. The main proteins involved in blood coagulation concentrated in platelets are von Willebrand factor (vWF), fibrinogen, and factor V. The mechanisms underlying their presence in cells with little or no protein synthesis capacity involve endogenous synthesis in the megakaryocyte, the bone marrow cell from which platelets are derived, endocytosis of plasma protein by megakaryocytes or platelets, or both mechanisms operating together. Because these proteins participate in the mechanisms of platelet–platelet and platelet–surface interactions, which occur where vascular integrity is compromised, their most important source is probably that released from the α granules through the open canalicular system, made accessible in a greater concentration to the nascent hemostatic plugs than the corresponding proteins in the plasma milieu.

The important pathophysiological role of intraplatelet coagulation proteins has been established by naturally occurring models—i.e., the inherited deficiencies or abnormalities of these proteins—which indicate clearly that platelet defects interact with plasma defects in determining the degree and severity of the bleeding disorders. von Willebrand disease (vWD) is the epitome of the inherited bleeding disorders in which the amount of platelet vWF, the protein deficient or defective in these patients, interacts with the plasma defects in determining the severity of the impairment of hemostasis. There is some preliminary evidence that deficiencies or abnormalities of platelet vWF also play a role in a few acquired bleeding disorders. Finally, platelet vWF is of therapeutic importance, because normal platelets must be transfused to correct defective hemostasis and stop bleeding in some cases.

Platelet vWF

vWF. vWF, a large glycoprotein encoded by a gene on chromosome 12, circulates in plasma at concentrations of 10 μg/ml as a noncovalent complex with coagulation factor VIII, the protein encoded by a gene on chromosome X that is deficient or defective in hemophilia A (1). This molecular complex is essential for normal survival of factor VIII, which is stabilized in the circulation, potentiated in its cofactor activity in intrinsic coagulation, and protected from inactivation by the naturally occurring proteolytic enzyme-activated protein C (1). The other important function of vWF in hemostasis is to support platelet plug formation at sites of vascular injury, by binding to the exposed subendothelium, forming a bridge between this surface and platelets (platelet–surface interactions) and causing platelet aggregation at high wall shear rates (platelet–platelet interactions) (1). These functions are facilitated by the peculiar structure of vWF, arranged in multimers of increasing size up to 20×10^6 daltons, built up from a subunit of $\approx 2.5 \times 10^5$ daltons, and by the presence or exposure on the platelet membrane of glycoproteins that function as receptors for vWF, the glycoprotein Ib, and the glycoprotein complex IIb/IIIa (1). vWF is synthesized in the vascular endothelial cell (2), the main source of the plasma protein (through constitutive or stimulated release from the luminal portion of the endothelial cell) and of the subendothelial protein (through abluminal release) (1). vWF is also synthesized by the megakaryocyte (3), as demonstrated by metabolic labeling studies (3) and detection of mRNA in these cells (4).

Platelet vWF. A large intraplatelet pool of vWF was first identified in 1974, representing as much as 15% of the total of this protein circulating in blood (5). The platelet factor is exclusively localized and stored in the α granules (3, 6) and does not exchange with plasma *in vitro* even after prolonged incubation (5, 7). *In vivo*, no platelet vWF was measurable after patients had been infused for a period exceeding the platelet life-span with cryoprecipitate, a source of exogenous vWF that had normalized low plasma levels in the recipient (7, 8).

Even though platelet and plasma vWF are products of the same gene, there are a number of structural and functional differences between the two proteins. The platelet protein contains a set of multimers of higher molecular weight than those in plasma (9–12). Another difference is that in plasma there is a relationship between blood group and vWF levels (group O individuals have lower levels than non-O individuals), but this relationship does not hold for platelet vWF (13).

Recently, the protein purified from platelets has been compared with that purified from plasma, showing differences and similarities (14). For instance, the apparent molecular mass of the subunit of reduced platelet vWF is similar to that of the corresponding plasma subunit (14). On the other hand, comparative analysis of carbohydrate composition revealed substantial posttranslational differences, the sialic acid and galactose contents of the platelet protein being approximately half those of the plasma protein (14). Perhaps the reduced sialic acid content of platelet vWF might explain why the protein is more susceptible to proteolysis than the plasma protein, since sialic acid protects from amino-terminal proteolytic cleavage (15). One important functional difference between platelet and plasma vWF is in their capacity to support platelet–vessel wall interactions. Both purified preparations bind to collagen with similar affinities (14). Platelet vWF binds with higher affinity than plasma vWF to the surface of thrombin-stimulated platelets (14, 16), after its release from the α granules and assembly on the platelet membrane following exposure of the glycoprotein complex IIb/IIIa receptor (17). This enhanced activity of platelet vWF, probably related to its supranormal multimeric structure (9, 10), should facilitate the concentration on the platelet membrane of the protein released from α granules (17), the formation of platelet aggregates in the presence of high shear forces (16), and eventually the arrest of bleeding from small vessels. That platelet vWF has a major function in supporting hemostatic plug formation is supported by the fact that in normal individuals platelet vWF is the main determinant of the skin bleeding time, more powerful than blood group, platelet count, hematocrit, age, or plasma vWF levels (13). On the other hand, purified plasma vWF binds to the platelet

glycoprotein Ib with higher affinity than the platelet vWF (14). Since the properties of plasma vWF reflect those of subendothelial vWF, because they both originate from the endothelial cell, it is mechanistically plausible that the higher affinity of subendothelial vWF for glycoprotein Ib facilitates the initial adhesion of platelets to the subendothelium exposed when blood vessels are transected (18, 19).

vWD. vWD is the most frequent inherited bleeding disorder, since mild forms occur in ≈1% of the general population (20). The disease is due to the deficiency or abnormality of vWF (21). vWF defects impair two main mechanisms of normal hemostasis: platelet plug formation, as a consequence of the poor or absent support by vWF of platelet–surface and platelet–platelet interactions, and fibrin formation, as a consequence of the poor or absent stabilization of coagulation factor VIII in plasma by vWF.

Typical laboratory expressions of vWD are low or unmeasurable plasma levels of vWF (assayed as immunoreactive protein or functional activity), a prolongation of the skin bleeding time, and low factor VIII (21). Clinical manifestations reflect the dual alterations of primary hemostasis and fibrin formation, with symptoms of mucosal bleeding (epistaxis, menorrhagia) accompanied by symptoms of postoperative and soft-tissue bleeding (hematoma, hemarthrosis) (21). The severity of clinical symptoms is, generally, roughly proportional to the degree of vWF deficiency in plasma, but there are exceptions to this general rule (see below).

In the absence of a comprehensive genetic classification, vWD is currently classified into three main phenotypes (22): type 1, the most common dominantly transmitted form, accounting for 70–80% of cases, characterized by concomitantly low levels of vWF measured as immunoreactive protein and functional activity; type 2, accounting for 15–20% of dominant or recessive forms, characterized by low levels of vWF activity contrasting with normal levels of antigen; and type 3, the rarest and most severe form of the disease (1 case in 1–2 million of the general population), usually transmitted recessively and characterized by unmeasurable vWF in the homozygous state (22). Data on the molecular pathology of the vWF gene available so far indicate that type 3 disease is usually related to relatively gross gene defects that markedly impair the expression of the protein (deletions, stop codons, cis defects in mRNA expression) (21); type 2 disease is usually associated with more subtle defects (missense mutations) that cause single amino acid substitutions resulting in a dysfunctional protein. Much remains to be done to unravel the molecular basis of type 1 vWD (21).

Platelet vWF in vWD

The pattern is very homogeneous in patients with type 3 vWD, with the protein unmeasurable even with highly sensitive immunoassays (7, 8, 23, 24) (Table 1). In contrast, vWF antigen is usually measurable in normal amounts in platelets from patients with type 2 (dysfunctional) vWD, but vWF activity is low or unmeasurable (8, 25). This discrepant pattern of vWF measurements generally mirrors that seen in plasma (normal antigen, low activity), although in a few patients, structural vWF abnormalities, seen in multimeric analysis, are more prominent in plasma than in platelets (9, 25) (Table 1). Finally, the pattern of platelet vWF is quite heterogeneous in type 1 vWD (25, 26), in striking contrast with the uniform plasma pattern (Table 1). The majority of patients have normal levels of immunoreactive protein and activity in platelets but low levels in plasma (25, 26). This subgroup of patients, called "*platelet normal*" (26), contrasts with a group characterized by corresponding low levels of vWF in plasma and platelets ("*platelet low*") (25, 26). Other patients have low or unmeasurable vWF activity in platelets but normal antigen ("*platelet discordant*") (26). Finally, a few individuals, not clinically affected and linked to vWD only because they are the heterozygous parents of type 3 patients, have a puzzling pattern, with vWF normal in plasma but low in platelets (25, 27). Due to current insufficient knowledge of the molecular defects underlying type 1 vWD, we can only speculate about the pathophysiological mechanisms that cause these varied phenotypes.

The platelet low phenotype is probably due to a global defect in the production of vWF (25, 26) (Table 1). The study of endothelial cells harvested and cultured from the umbilical vein of a newborn girl (28) showed that her endothelial cells contained less vWF than normal cells by immunofluorescence and released into the culture medium smaller amounts of the protein after stimulation with agonists such as thrombin and calcium ionophore (28). There were also smaller amounts of vWF in the extracellular matrix of the patient's endothelial cells, and these supported platelet adhesion poorly upon perfusion with normal blood (28). Low vWF in endothelial cells, subendothelial matrix, platelets, and plasma would indicate that the defect in type 1 platelet low is a reduced rate of production, storage, and release of functional vWF.

In the phenotype platelet normal, the plasma defect is perhaps secondary to a cellular defect (not necessarily related to a defect of the vWF gene) that in resting conditions determines reduced release of a functional vWF molecule (Table 1). This hypothesis is indirectly supported by the occurrence of a massive release of vWF in these patients after i.v. infusion of the vasopressin analogue desmopressin (26, 29). No endothelial cell studies have been done so far in well-characterized patients with the platelet normal phenotype. However, in two patients with type 1 vWD, upon stimulation little vWF was released from their cultured endothelial cells, even though immunofluorescence revealed that the protein was apparently contained in normal amounts (30). Platelet vWF was not measured, so we do not know whether or not these patients belong to the platelet normal phenotype (30).

Finally, there is at the moment little understanding of the platelet discordant phenotype and of the phenotype characterized by low platelet/normal plasma vWF. In the former, the discrepancy between platelet vWF antigen (normal) and activity (low), as well as the large increase in antigen contrasting with little or no change in activity after desmopressin infusion, would suggest that the defect is due to the presence of a normally released dysfunctional molecule (26) (Table 1). Patients with the phenotype platelet low/plasma normal, described in at least six different cases (25, 27), respond poorly to desmopressin in terms of plasma vWF (31), consistent with the fact that the response to desmopressin is usually proportional to platelet vWF levels (26). Perhaps the platelet low/plasma normal phenotype has a defect of vWF stabilization in

Table 1. Pathophysiology of vWD

	vWF		Response to desmopressin	Pathophysiology of the defect	Molecular defects
	Plasma	Platelet			
Phenotype 1					
Platelet low	Low	Low	Low	Low production of normal vWF	Unknown
Platelet normal	Low	Normal	Normal	Defective release of normal vWF	Unknown
Platelet discordant	Dysfunctional	Dysfunctional	Normal	Normal release of dysfunctional vWF	Unknown
Phenotype 2	Dysfunctional	Dysfunctional	Normal	Normal release of dysfunctional vWF	Missense mutations
Phenotype 3	Unmeasurable	Unmeasurable	Absent	Absent vWF production	Gross gene defects

the α granules, causing its abnormal susceptibility to platelet proteases.

Significance of Platelet vWF in vWD

The varied patterns of platelet vWF observed in vWD have important clinical implications, because experimental and clinical data indicate that defects of the platelet protein are important in determining the degree of the impairment of primary hemostasis.

Perfusion Experiments. *In vitro* experiments devised to investigate how flowing blood of patients with vWD interacts with the subendothelium were originally based upon perfusion at high shear rates with the patient's anticoagulated blood of deendothelialized human umbilical arteries or artificial flow chambers whose glass surfaces had been coated with fibrillar collagen, an adhesive surface mimicking the subendothelium of injured vessels (32, 33). Early experiments with citrated whole blood as perfusate established that platelet adhesion is low in vWD (34) but could not discriminate between the relative contributions of platelet and plasma vWF in determining the adhesion defect. Subsequently, to try to differentiate these contributions, perfusates of reconstituted blood containing normal red blood cells and four different mixtures of washed platelets and citrated platelet-poor plasma were compared: normal platelets and normal plasma; platelets and plasma from patients with type 3 vWD, containing no measurable vWF; normal platelets and patient plasma; patient platelets and normal plasma (35). The marked defect of platelet adhesion when the perfusate contained patient platelets and plasma was improved when normal platelets replaced patient platelets and, to a greater extent, when normal plasma replaced patient plasma (35). These results indicate that even though normal concentrations of plasma and platelet vWF are necessary for optimal platelet adhesion, platelet vWF can partially support adhesion even in the absence of plasma vWF. The contribution of platelet vWF to support platelet adhesion was confirmed by other perfusion experiments with citrated blood from patients with low plasma vWF but platelet vWF levels ranging from unmeasurable to normal (36). There was a close correlation between platelet adhesion and plasma vWF in patients with low or unmeasurable platelet protein, but adhesion was much higher than expected from plasma vWF levels in patients with normal platelet vWF (36). The same patients were subsequently restudied *ex vivo*, with a perfusion system based upon flowing native, non-anticoagulated blood drawn directly from a forearm vein over the adhesive surface (37). With this system one can explore platelet–surface interactions during the generation of thrombin and fibrin formation, which cannot be evaluated when anticoagulated blood is used as perfusate (37). In this more "physiological" system, platelet adhesion was impaired only in patients with very low platelet vWF activity (type 3, platelet discordant and platelet low), whereas it was similar to that seen in normal individuals in platelet normal patients (37). In addition, platelet vWF had a clear effect on the volume and height of platelet plugs, which were much less reduced in patients with normal platelet vWF than in those with low levels (37). On the whole, platelet plug formation was impaired in all patients with vWD, even when platelet adhesion was normal, but normal platelet vWF helped to partially compensate the plasma defect.

Animal Experiments. Additional evidence for a role of platelet vWF in primary hemostasis stems from experiments of bone marrow transplantation carried out in pigs with a form of vWD that in the homozygous animal is very similar to that of human type 3 disease (38). In an early study of an affected animal transplanted with bone marrow from a normal pig that produced platelets containing normal amounts of vWF, the bleeding time was slightly but inconsistently shortened post-transplantation, from values >15 min to values varying between 5 and >15 min (normal values in normal pigs: 2.2 ± 0.75 min) (39). Nichols *et al.* (40) have extended those experiments and this issue of the *Proceedings* report the results of a series of crossed trasplantations carried out in normal and vWD pigs. Bone marrow grafts generated two types of chimeric animals, one with normal platelet vWF factor but lacking the plasma protein and another lacking platelet vWF but with normal plasma protein. In the chimeric vWD animals, whose vWF is restricted to platelets and lacking in endothelial cells, subendothelium, and plasma, the bleeding time was >15 min, as in the wild-type animal. In the chimeric normal animal, whose vWF is restricted to plasma, endothelial cells, and subendothelium and lacking in platelets, the bleeding time was slightly longer than in the wild-type normal animals (6.1 ± 2.2 min vs. 3.8 ± 1.5 min). These elegant experiments in pigs reinforce and extend a few important points already established by the *in vitro* and *ex vivo* perfusions in humans. Platelet vWF is necessary for normal primary hemostasis, because its absence in chimeric normal pigs determines a slight but definite prolongation of the bleeding time, but not sufficient, because its presence does not shorten the bleeding time very much, if at all, in chimeric vWD animals with no vWF in plasma, endothelial cells, and subendothelium.

Clinical Data. In vWD, a number of clinical and laboratory abnormalities cannot be explained solely by the degree of plasma vWF defects. For instance, not all patients have a prolonged bleeding time, the simplest laboratory marker of defective platelet plug formation; even when prolonged, the bleeding time is not always proportional to the defect of vWF measured in plasma as immunoreactive protein or as functional activity (21). Another unexplained observation is that replacement therapy with fractions rich in vWF (such as cryoprecipitate and factor VIII concentrates) normalize the bleeding time poorly and transiently, notwithstanding the attainment of normal postinfusion vWF levels (41, 42).

When vWF was measured in platelets in parallel with plasma vWF, the erratic bleeding time patterns became clearer. It was seen, for instance, that patients with type 1 vWD with normal platelet vWF (platelet normal) often have normal or slightly prolonged bleeding times, notwithstanding plasma vWF as low as 10–20% of normal (26). On the other hand, patients belonging to the platelet low phenotype have consistently prolonged bleeding times, even when their plasma vWF is similar to or higher than that in the platelet normal patients (26). Finally, the correlation coefficient between platelet vWF activity and the bleeding time is high ($r = +0.80$), whereas there is little correlation between the latter and plasma vWF ($r = +0.17$) (43). Obviously, these observations are not meant to indicate that a defect of platelet vWF is the only or main determinant of the bleeding time prolongation, because the few individuals who carry the phenotype of low platelet/normal plasma vWF have normal bleeding times and do not bleed excessively (25, 27). Yet, natural human models of platelet–plasma vWF compartmentalization, such as the platelet normal and platelet low phenotypes, *in vitro* and *ex vivo* perfusion experiments with the blood of these patients, and the cross-transplantation animal experiments have together firmly established that the integrity of both vWF compartments is essential to fully secure primary hemostasis.

Therapeutic Implications. Usually, patients with vWD are treated with plasma fractions that contain factor VIII and vWF, the proteins low or unmeasurable in these patients (21). Many of them, however, can be effectively treated with desmopressin, which raises the plasma levels of factor VIII and vWF by releasing these proteins from endogenous stores (29). Desmopressin is cheaper than plasma fractions and completely avoids the risk of transmission of blood-borne viruses still associated with the use of plasma fractions despite the recent adoption of virucidal methods. Both treatments are usually effective in increasing and often normalizing low plasma levels of factor VIII and vWF. Yet, it has been known for many years that in some patients there is not always the correction of the bleeding time after either treatment (21).

For patients with type 3 disease, unresponsiveness to desmopressin is probably due to the complete deficiency of vWF in plasma, in cells, and hence in storage sites. For patients with type 1 disease, unresponsiveness is usually mirrored by the levels of vWF in platelets, nonresponders being those with low or dysfunctional platelet vWF (platelet low and discordant) (26). This does not imply that the protein is actually released from platelets by desmopressin but, rather, that platelet vWF appears to mirror the status of the endothelial cell stores from which the protein is released into plasma.

The correction of the prolonged bleeding time after the infusion of such plasma fractions as cryoprecipitate and factor VIII concentrates is often poor and never sustained (41, 42, 44). Structural and functional vWF abnormalities in factor VIII concentrates, reflected by an abnormal multimeric structure and other abnormalities (44), do not explain this failure fully, because fractions with an intact multimeric structure are not clearly more effective than those lacking high molecular weight multimers (41, 42). Again, awareness of the important role of platelet vWF in supporting primary hemostasis provided a clue to understanding these discrepancies (45, 46). Infusion studies in patients with type 3 disease showed that, whereas cryoprecipitate alone was insufficient for normalizing the bleeding time, the subsequent administration of normal platelets (in amounts similar to those used for patients requiring platelet transfusions for thrombocytopenia) normalized the bleeding time consistently and in a sustained fashion (45). Most importantly, mucosal hemorrhages, not controlled by cryoprecipitate alone, stopped after platelet infusion (45). Additional studies demonstrated that even though the infusion of normal platelets alone (to reach one-fifth of the platelets circulating in the recipient) did shorten the prolonged bleeding time from >30 min to 14–17 min, this value did not become normal unless cryoprecipitate was also infused (46), and that the bleeding time became normal only when plasma vWF activity reached 14–18% of normal values (46). These results are strikingly consistent with the observations made in the natural model of patients with type 1 platelet normal, in whom the presence of platelets with normal vWF was sufficient to support normal or near-normal bleeding times provided plasma levels were at least 10–20% of normal (26).

These findings should not be taken to indicate that platelet concentrates are always needed in addition to plasma fractions. Coagulation factor VIII is always fully corrected by the infusion of plasma fractions containing it (41, 42, 44), and the normalization of fibrin formation is usually sufficient to stop soft-tissue and to prevent surgical bleeding, which are mainly determined by the degree of the factor VIII defect (47–49). On the other hand, hemorrhages in mucosal tracts (gastrointestinal bleeding, severe menorrhagia) and in the central nervous system may not be stopped unless the bleeding time defect is fully corrected (50-52). In these instances, the addition of platelet concentrates is warranted.

Platelet vWF in Acquired Bleeding Disorders

vWF is present in normal amounts in platelets from patients with the acquired von Willebrand syndrome (53). There is no vWF gene defect in this syndrome and plasma defects are due to vWF removal from plasma, through mechanisms such as autoantibodies, nonspecific complex formation with immunoglobulins, and adsorption to tumor cells (53). Most patients with the acquired von Willebrand syndrome have normal or borderline bleeding times, particularly when plasma levels are not too markedly reduced. Hence, normal levels of platelet vWF clearly help to compensate for the plasma defects in this condition. On the other hand, there are examples of acquired conditions with defective platelet vWF that may contribute to abnormal hemostasis. A reduction of platelet vWF was found, for instance, in patients with uremia who had prolonged bleeding times and normal to high plasma vWF levels (54). Subsequently, however, normal platelet vWF was found in other uremic patients, whatever their bleeding times (55). An isolated defect of platelet vWF was found in a boy with lactoferrin deficiency, impaired granulocyte function, and recurrent bacterial infections (56). The boy, who had undue bleeding after minor surgery and a bleeding time varying from normal to slightly prolonged, had low platelet vWF and an abnormal multimeric structure but no significant plasma deficiency (56). Interestingly, his defects were less prominent in platelets collected in the presence of protease inhibitors, suggesting that they may be due, in part or totally, to excessive platelet proteolysis, whether due to an intrinsic abnormality of the platelet proteolytic enzyme system or to the presence of an abnormal vWF factor molecule highly susceptible to physiological proteolysis. Platelet vWF may be involved in the bleeding tendency of patients with myeloproliferative disorders, especially essential thrombocythemia, but also with polycythemia vera. Some patients have low platelet vWF activity, usually associated with higher or normal values of vWF antigen, whereas plasma levels are normal or modestly decreased (57-59). There may also be structural abnormalities of platelet vWF, with loss of large molecular weight multimers (59). The quantitative and qualitative abnormalities paralleled the presence of a history of bleeding symptoms but were not correlated with bleeding time prolongation (59). Though these findings need confirmation in a larger series, it can be surmised that the clonal abnormalities present in thrombocythemia lead to the production of an abnormal vWF by the megakaryocyte and/or that abnormal platelets excessively proteolyze and inactivate a normally produced vWF. In myeloproliferative disorders, the qualitative and quantitative abnormalities of platelet vWF are much less prominent in plasma, a milieu less rich in unimpeded proteolytic enzymes.

Conclusions

In vWD, platelet vWF interacts with plasma vWF in determining the degree of the abnormalities of platelet–vessel wall interactions, reflected *in vivo* by such diagnostic tests as the skin bleeding time. It is difficult to establish which vWF compartment is more important: chimeric vWD pigs, with normal platelet vWF but low plasma levels, have very prolonged bleeding times; on the other hand, the closest human equivalent of this situation—i.e., patients with normal platelet vWF but low plasma vWF—have normal or borderline bleeding times (26). Perhaps platelet vWF can compensate, in part or fully, for the plasma defect only when this is not too severe (>10–20% of normal) but not when vWF is unmeasurable, as in pigs and humans with type 3 vWD. Furthermore, normal levels of plasma vWF are not sufficient to support normal bleeding time, as shown experimentally by the prolonged bleeding times of chimeric normal pigs with low platelet vWF and by the poor correction of bleeding time in type 3 patients treated with plasma fractions (41, 42, 44), unless at least one-fifth of their circulating platelets contains normal amounts of vWF (46). Finally, platelet vWF may play a role not only in congenital bleeding diseases but also in acquired conditions such as uremia and myeloproliferative disorders, so it should be considered as one possible cause of acquired bleeding when plasma levels are normal.

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