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# Biology of TGF-β in knockout and transgenic mouse models

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**Biology of TGF-β in knockout and transgenic mouse models.** This paper reviews the basic biology and biochemistry of the TGF-β isoforms including their unique serine-threonine receptors and signaling intermediates. Dysregulation of TGF-β expression and/or receptor/signaling function have been implicated in a wide variety of pathologies. We will discuss mechanisms underlying some of these disease processes as gained from study of transgenic mice in which expression of TGF-β1 has either been lost by targeted deletion of its gene, is overexpressed in a tissue-specific manner, or blocked by its latency associated peptide.

## Physiology and pathophysiology of TGF-β and its receptors

Transforming growth factor-β (TGF-β) is a multifunctional cytokine with effects on nearly every tissue and cell type [1, 2]. Three distinct isoforms of the peptide are found in mammalian species and expressed in unique patterns, although each isoform appears to signal through the same set of cell surface receptors [3–5]. This suggests that isoform-specific effects lie upstream of the signaling receptors and likely involve differential activation of the promoters as well as possible differences in extracellular localization, trafficking, and activation from their respective latent forms. Principal effects of TGF-β on cells include inhibition of the growth of hematopoietic, epithelial, and endothelial cells, stimulation of chemotaxis of cells including lymphocytes, macrophages, and fibroblasts, and stimulation of matrix protein production by mesenchymal cells [6]. The most concentrated source of TGF-β is that of the type 1 isoform in platelet alpha granules consistent with its critical roles in repair of soft tissue and in fracture healing [7]. Dysregulated expression of or response to TGF-β has been implicated in a wide variety of disease processes including autoimmune disease [8, 9], fibrotic disease and chronic inflammation [10, 11], parasitic disease [12], neurodegenerative disease [13], and carcinogenesis [14, 15]; in almost every case this dysregulation involves the type 1 isoform of TGF-β consistent with predictions based on analysis of its promoter [16].

### *TGF-βs signal through serine-threonine kinase receptors*

In contrast to many mitogenic growth factors that signal through receptors having intrinsic tyrosine kinase activity or that collaborate intracellularly with tyrosine kinases, most members of the TGF-β superfamily of ligands signal through a multimeric receptor complex consisting of type I and type II receptors, each of which has intrinsic serine-threonine kinase activity [3–5]. Signal transduction is initiated by a sequence of events involving: (1) binding of the ligand to the type II receptor; (2) recruitment of the type I receptor to the liganded type II receptor complex; (3) transphosphorylation of a glycine-serine-rich domain (GS domain) of the type I receptor by the type II receptor; and (4) propagation of the signal (Fig. 1) [17]. The signal transduction pathways have yet to be elucidated in any detail. However, in recent months there has been an explosion of research focused on a family of at least six mammalian proteins related to *Drosophila* Mad (Mothers against dpp), a cytoplasmic protein shown by genetic analysis to lie downstream of a TGF-β-family ligand called decapentaplegic (dpp) [18, 19]. Proteins belonging to this family have no known structural or functional motifs, but have been shown to mediate developmental signals from TGF-β superfamily ligands in not only *Drosophila*, but also in *C. elegans* and *Xenopus* [20, 21]. A pattern is now emerging from studies in both *Xenopus* and mammalian cells suggesting that certain of these proteins, or perhaps complexes of these proteins, mediate signals in a ligand-specific manner, in the process becoming phosphorylated and translocated from cytoplasm to nucleus (Fig. 1) [22–24].

### *Loss of TGF-β responsiveness may be common to a variety of pathologies*

There is now substantial evidence suggesting that the TGF-β receptors and downstream signaling pathway(s) constitute a tumor suppressor pathway and that loss of either the receptors or signaling intermediates is associated with carcinogenesis [15]. Specific examples include loss of TGF-β type II receptors by a mechanism called microsatellite instability [25] and involving permutation of a polyadenine tract in the coding region of the receptor in patients having genetic defects in DNA repair mechanisms [26], mutational inactivation of type II or type I receptor kinase activity [27, 28], or by mutations resulting in a dominant negative effect [29]. The observation that microsatellite mutation of the type II receptor is common in some but not in other cancers characterized by replication error defects, demonstrates that receptor loss is a selected trait in certain cancers, such as colon and gastric carcinoma, in which it confers a growth advantage [30]. Preliminary studies in our laboratory also demonstrate that loss of responsiveness to TGF-β in transgenic mice expressing a dominant negative type II receptor predisposes to spontaneous and experimentally-induced carcinogenesis, showing that it contributes directly to tumor progression (E. Böttinger and L. Wakefield, personal communication). Recently, two of the Mad family proteins specifically implicated in signaling from TGF-β ligands, Madr2 and dpc4 (also called Smad2 and Smad4), have been shown to be candidate tumor suppressor genes in that they are deleted in certain colon, breast, and pancreatic cancers

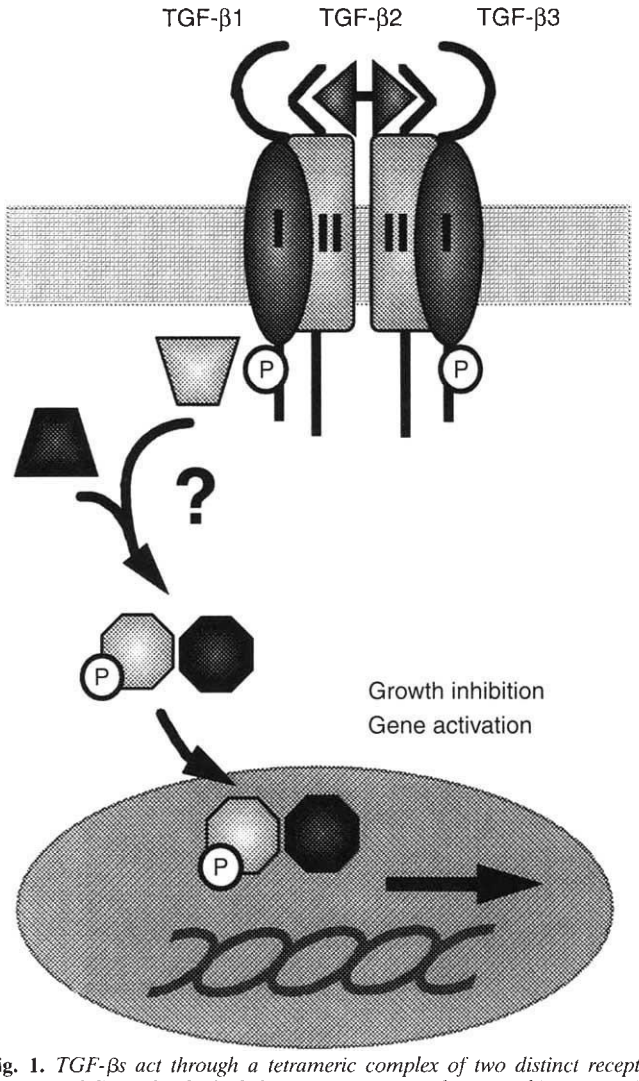


**Fig. 1.** *TGF-βs act through a tetrameric complex of two distinct receptor types I and II, each of which has intrinsic serine-threonine kinase activity.* Ligand-activation of the receptor complex leads to phosphorylation of the type I receptor by the type II receptor kinase. Signal transduction is mediated, at least in part, via cytoplasmic proteins belonging to the Mad family. Data show that Smad3 (lighter shading) is associated with the receptor complex [34] and that Smad4/DPC4 (darker shading) is also necessary for signal transduction from TGF-β [34]. Smad2 [32] and Smad3 [34] are phosphorylated and translocated to the nucleus following treatment of cells with TGF-β. In the hypothetical scheme shown here, it is proposed that different Smad proteins may form homodimeric or heteromeric complexes. The mechanisms whereby these proteins lead to gene activation are currently unknown.

[31–33]; introduction of dpc4 into null tumor cells has been shown to restore TGF-β signaling [34] (M. deCaestecker and R. Lechleider, personal communication). Taken together, the data demonstrate that TGF-β signals through a tumor suppressor pathway.

While escape from negative growth regulation by TGF-β now appears to be common to many different cancers, this mechanism has not been definitively implicated in other pathologies. However, preliminary studies in our laboratory suggest that a similar mechanism may be involved in neurodegenerative diseases (K. Flanders, personal communication).

### *Dysregulation of TGF-β ligands in disease*

Loss of TGF-β signaling is frequently associated with increased expression of TGF-β1. In carcinogenesis, this results in double jeopardy. The tumor cell is no longer suppressed by autocrine action of the TGF-β it secretes, allowing paracrine effects of the secreted TGF-β to promote tumor growth indirectly by suppressing immune surveillance, increasing desmoplasia, and enhancing angiogenesis [14]. Moreover, as might have been predicted from the strong effects of TGF-β on regulation of extracellular matrix protein production, there is now an extensive body of literature demonstrating overproduction of TGF-β1 in a host of fibrotic diseases including glomerulonephritis, pulmonary fibrosis, and liver cirrhosis [reviewed in 10]. Blocking the TGF-β in experimental models of fibrosis ameliorates the symptoms, suggesting a critical role for TGF-β in the disease process [35, 36]. On the other hand, deficiency of TGF-β1 leads to defects in immune cells as seen in experimental models of autoimmune disease [7, 8, 37]. Thus, in both allergic experimental encephalomyelitis, a model of multiple sclerosis, and in experimental arthritis, injection of TGF-β1 suppresses symptoms of disease, whereas injection of antibodies to TGF-β1 exaggerate the process [7, 8]. This review discusses the use of transgenic and knockout mouse models in which TGF-β1 is either overexpressed or deficient, with an emphasis on new insights that these models have provided into the role of TGF-β1 in clinical disease.

## Use of transgenic and knockout mouse models to study effects of dysregulation of TGF-β1

### *TGF-β1 knockout mice: Implications of their phenotype*

As noted above, TGF-β1 appears to be the predominant isoform underlying numerous pathologies, including carcinogenesis, fibroproliferative, parasitic, and autoimmune diseases [6, 10]. With the development of gene targeting strategies, truly isoform-specific activities are now gaining a more clear definition through models in which the expression of each individual isoform has been disrupted [38–41]. Indeed, while the phenotype of the TGF-β3-deficient mouse has defined this isoform as a critical participant in the processes of palatogenesis and pulmonary maturation during development, the initial evaluations of TGF-β1-deficient mice have subtantiated the importance of this isoform in the maintenance of immunological homeostasis [38, 39, 42, 43]. In fact, these models clearly show that the isoforms are not functionally redundant *in vivo*, and that selective loss of each isoform yields a severe phenotype.

The predominant phenotype of the TGF-β1 knockout mouse suggested the loss of a critical regulator of immune function. The phenotype is best characterized as an excessive inflammatory response, with a massive infiltration of leukocytes (principally lymphocytes and macrophages) in several organs. Heart, lung, liver, salivary gland, pancreas, stomach, and intestine are uniformly involved, with occasional involvement of brain, kidney, and skeletal muscle. The syndrome develops rapidly, beginning during the first week of life and resulting in severe wasting and death by the fourth week of life [38, 39, 42].

The typical pattern of tissue infiltration in TGF-β1-deficient mice is perivascular in nature. It appears to evolve from the earliest lesions, which are primarily defined by the increasing adhesion of leukocytes to vascular endothelium, with ultimate perivascular accumulation. The profound immune dysregulation

that develops in the absence of TGF-β1 gene expression is also associated with an apparent alteration in the control of normal hematopoiesis. The latter is a component of the phenotype that is enhanced in backgrounds that obviate the evolution of the inflammatory process [44], and may be linked to the loss of cell cycle controls normally exerted by TGF-β1.

An emerging consensus regarding the predominant phenotype is that it represents a unique and important model of autoimmunity. In many other murine models of autoimmune disease, including in the MRL/*lpr* model of systemic lupus erythematosis (SLE) and murine experimental allergic encephalomyelitis (EAE) [8, 45], endogenous production of TGF-β1 is seen as a natural host reponse to the ongoing inflammatory process. Another feature of the TGF-β1 knockout mouse phenotype that is highly suggestive of an autoimmunine basis for the phenotype is the appearance of enhanced expression of antigens of the major histocompatibility (MHC) class I and class II genes within several peripheral tissues, first detected during the neonatal period [46]. Susceptibility to several autoimmune diseases has been linked to MHC class II genes, as in insulin-dependent diabetes mellitus (IDDM) and pemphigus vulgaris in humans, and in the NOD mouse model of IDDM [47–49]. Moreover, our recent work now documents the critical role of class II molecules in the pathogenesis of this phenotype, as it is absent in the CD4+ T cell-deficient, MHC class II antigen-negative background [44].

Collectively, these data suggest that the absence of TGF-β1, as in the TGF-β1 null mice, might lead to an autoimmune process. Other phenotypic features consistent with this interpretation are the presence of SLE-like serum autoantibodies to nuclear antigens, immune complex deposits in renal glomeruli, and a progressive lymphocytic tissue infiltration similar to that present in human autoimmune syndromes such as Sjögrens [37, 50]. These model systems now provide a unique tool for the study of renal pathologies typically associated with chronic immune disorders.

### *Overexpression of TGF-β1 under control of the albumin promoter*

In the kidney, local production of TGF-β either by intrinsic renal cells or by infiltrating inflammatory cells has been implicated as a key mediator of tissue fibrosis [10]. In addition, up-regulation of TGF-β receptor expression as recently observed in experimental glomerulonephritis [51, 52] is in keeping with a model of enhanced activity of the entire TGF-β signaling cascade in tissue repair and remodeling.

There is now growing evidence that circulating TGF-β1 may have important effects in physiologic and pathophysiologic processes. Expression of a TGF-β1 transgene under the control of an albumin promoter/enhancer (Alb/TGF-β1) in the liver of transgenic mice resulted not only in hepatic fibrosis, but was also notable for myocarditis, atrophy of testis and pancreas, and renal disease [53]. Renal lesions correlated well with increased levels of circulating TGF-β1 in different transgenic lines and were characterized by progressive mesangial expansion, accumulation of glomerular immune deposits and matrix proteins, and interstitial fibrosis [54]. Glomerular deposits lacked complement and distributed predominantly in subendothelial and mesangial locations. Renal failure with nephrotic syndrome was fatal in one quarter of severely affected transgenic mice. These findings indicate that chronically elevated circulating levels of TGF-β1 induce progressive glomerulosclerosis. Interestingly, local expression of TGF-β1 and TGF-β3 mRNAs in diseased transgenic kidneys did not differ from nontransgenic control kidneys (M. Mozes, J. Kopp, personal communication) pointing to a direct pathogenetic role for circulating TGF-β1.

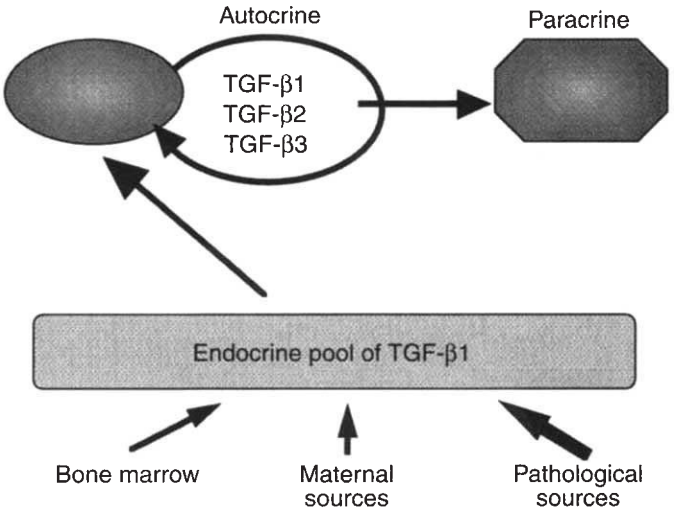


**Fig. 2.** *TGF-β1 is the only isoform of TGF-β involved in endocrine trafficking.* Action of TGF-β2 and TGF-β3 is restricted to local autocrine and paracrine modes. The sources of endocrine TGF-β1, the molecular forms of the complexes, and the mode of targeting to cells and tissues are presently unknown. Specific pathologies associated with increased levels of TGF-β1 in plasma suggest that the molecular "addresses" or chaperones of TGF-β1 secreted into the plasma from pathological sources such as tumor cells may differ from those of physiological levels of the protein, resulting in distinct targeting associated with the specific disease.

### *Physiological and pathological roles of endocrine trafficking of TGF-β1*

Evidence for a physiological role of circulating TGF-β1 comes from the unexpected discovery in TGF-β1(−/−) mice that TGF-β1 is transferred from heterozygous mothers to their TGF-β1(−/−) progeny [55]. TGF-β1 was transferred both transplacentally and lactationally to fetuses and neonates, respectively, localizing in a tissue-specific manner to give an immunohistochemical staining pattern in newborn TGF-β1(−/−) pups similar to that of wild-type littermates. This transferred protein plays a critical role in embryogenesis in that TGF-β1 null pups born to a TGF-β1 null mother uniformly displayed severe developmental defects in the heart [55]. The particular carrier protein(s) involved in this endocrine trafficking of TGF-β1 are not known, although a relevant example may be the transfer of TGF-β1 bound to IgG that is taken up by macrophages via Fc receptors and delivered in an active form to TGF-β receptors on interacting lymphocytes [56, 57].

The presence of physiologically significant levels of TGF-β1 in plasma of normal human subjects suggests that endocrine trafficking of TGF-β1 may play an important, yet undiscovered physiological role [58]. Again, the physiological form of the circulating TGF-β1 is unknown, although, based on the half-life of only two to three minutes for active TGF-β, it is likely that the TGF-β1 is in the latent form or complexed to a molecule such as thrombospondin [58]. While the source of plasma TGF-β1 is not known, preliminary evidence from the TGF-β1 null mice indicates

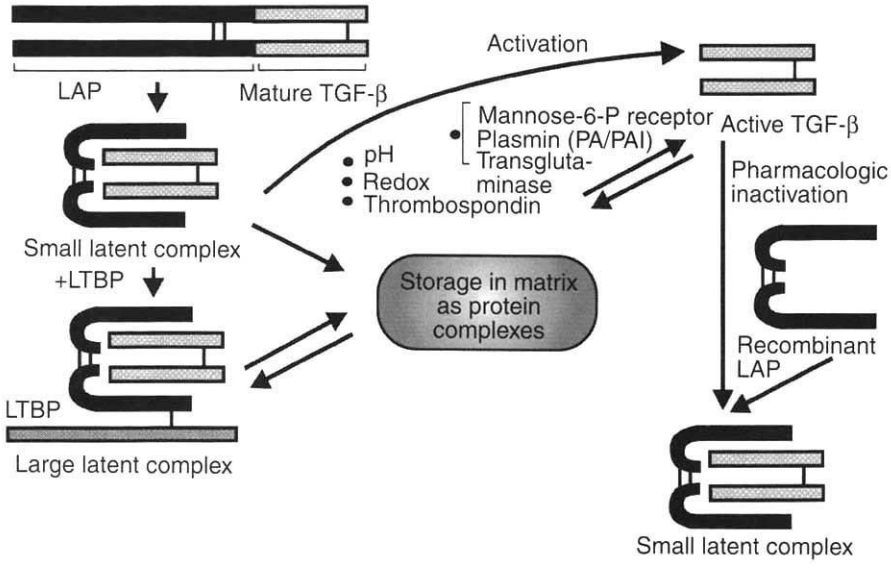


**Fig. 3.** *TGF-β precursors are proteolytically cleaved into the proregion (latency-associated peptide or LAP) and mature TGF-β.* LAP and mature TGF-β are noncovalently associated and secreted as biologically inactive complexes. "Small latent complexes" consist of LAP and mature TGF-β. Latent TGF-β binding proteins (LTBPs) may be covalently linked to small latent complexes to form "large latent complexes". Activation of latent TGF-β leads to release of active TGF-β from latent complexes. Proposed mechanisms of activation include acidic pH, redox, thrombospondin, mannose-6-P receptor, transglutaminase and proteolytic cleavage by plasmin [reviewed in 66]. Recombinant LAP can reassociate with active TGF-β inactivating its biological activities *in vitro* [68] and *in vivo* [69].

that it may be derived from bone marrow (J. Letterio, unpublished observation). Importantly, this mode of trafficking is unique to the type 1 isoform; plasma levels of TGF-β2 and TGF-β-3 are undetectable, suggesting that they are restricted to more localized autocrine and paracrine modes of action (Fig. 2).

Several reports now suggest that endocrine trafficking of TGF-β1 may not only be important in normal physiology, but also that dysregulation of endocrine trafficking, as evidenced by either excessive or deficient levels of TGF-β1 in plasma, may predispose to or be indicative of certain pathologies. As examples, elevated plasma TGF-β1 has been shown to be the best marker of invasive prostatic adenocarcinoma [59] and, in breast cancer patients, has been found to be a strong positive predictor for lethal fibrotic sequelae in liver and lung following bone marrow transplantation [60]. Moreover, elevated serum levels of TGF-β1 are present in thrombotic thrombocytopenic purpura [61]. As for physiological levels of plasma TGF-β1, the molecular forms of excess TGF-β1 deposited into the plasma by cells such as tumor cells are unknown [62]. However, the specific tissue targeting of this excess circulating TGF-β1 suggests that it might have a unique chaperone resulting in a "molecular address" distinct from that of basal levels.

In contrast, deficient levels of plasma TGF-β1 show a strong correlation to atherosclerosis. Patients with no vascular disease had plasma levels of TGF-β1 in the normal range, whereas patients having previously been diagnosed with significant stenoses of all three major coronory vessels all showed severe deficiencies in plasma TGF-β1 levels [63]. Interesting in this regard is that one of the genetic loci of hereditary hemorrhagic telangiectasia has been shown to be endoglin [64], a molecule known to bind TGF-β1 and facilitate its interaction with its receptors on endothelial cells [65]. Taken together, these two lines of evidence suggest that either a deficiency of TGF-β1 or of its ability to signal may impair physiological repair of blood vessel injury and predispose to vessel disease. The recent backcross of the TGF-β1 null mice into the nude background is now providing longer-lived mice in which this aspect can be investigated (J. Letterio, unpublished observations).

These observations in transgenic mouse models and in human disease suggest that it will be important to define to what extent circulating TGF-β1 participates in the pathogenesis of chronic fibrotic and proliferative diseases. In this context, understanding of the physical form of circulating TGF-β1, its source and regulation, as well as mechanisms of tissue sequestration will be essential.

### *Latency-associated protein inhibits TGF-β1 activity in vitro and in vivo*

The three mammalian isoforms of TGF-β (TGF-β1, TGF-β2 and TGF-β3) are secreted as latent complexes and activated extracellularly with release of the active cytokines from their noncovalently associated proregions, also known as latency-associated peptides (LAP) (reviewed in [66]; Fig. 3). The proregion of TGF-β1 (aa 30-278) [67], when independently expressed, associates noncovalently with active TGF-β, inactivating its biological activities *in vitro* [68]. We have examined whether systemic administration of recombinant TGF-β1 LAP would decrease the activity of excess active TGF-β1 *in vivo*. The neutralizing activity of recombinant LAP was assessed in a short-term assay measuring hepatocyte proliferation *in vivo* in Alb/TGF-β1 transgenic mice with elevated levels of bioactive TGF-β1 in the liver. Treatment with LAP reversed suppression of the early proliferative response induced by TGF-β1 in remnant livers after partial hepatectomy, indicating neutralization of the antiproliferative activity of elevated TGF-β1 during liver regeneration [69]. Studies of tissue distribution of radioiodine-labeled LAP showed accumulation of comparable levels of LAP in most parenchymal organs including liver, lung and kidney. Of interest, our *in vitro* studies indicated that TGF-β1 LAP inhibits active TGF-β2 and TGF-β3 with equal potency when compared with active TGF-β1 [69]. Hence, TGF-β1 LAP may be a potent inhibitor of all three TGF-β isoforms. It will be important to examine whether the promising TGF-β inhibitory activity of LAP as demonstrated in the short-term liver regeneration assay can be sustained in more chronic disease models. In this context, we are currently studying the effects of chronic

administration of recombinant LAP on the development of fibrotic kidney disease in Alb/TGF-β1 transgenic mice.

## Future issues

New insights into the role of TGF-β in biology and pathobiology have resulted from study of the TGF-β1 null and TGF-β1 transgenic mice. Key observations such as striking autoimmune processes in TGF-β1 null mice or induction of fibrotic disease by circulating TGF-β1 in Alb/TGF-β1 transgenic mice have not been predicted. Thus, a better understanding of the *in vivo* biology of TGF-β is imperative to enable design of safe and effective therapeutic strategies targeting the TGF-β system.

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