Pelvic organ prolapse (POP) is a disabling disorder in women characterized by a loss of pelvic floor support leading to the herniation of the uterus into or through the vagina. POP is a complex problem that likely involves multiple mechanisms, and available therapies are limited. In this issue of the JCI, Budatha et al. explore the dual role carried out by fibulin-5 in facilitating the assembly of normal elastic fibers and inhibiting MMP-9 activity, revealing a new mechanism critical to the maintenance of pelvic organ support.

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Pelvic organ prolapse (POP) is a major cause of morbidity among women in the United States. It occurs in approximately 35% of the adult female population, and the incidence increases with age (1). The impact of POP on women is significant, including sexual dysfunction, social isolation, depression, and poor body image (2). The etiology is multifactorial, with a history of vaginal childbirth being the most notable risk factor (3, 4). It is estimated that 11%–19% of women will require surgical repair in their lifetime to treat prolapse, and approximately one-third of these will require additional surgery for recurrent disease (5); thus it is important to define the molecular and cellular mechanisms underlying these conditions.

results in two alternate alleles (19). The insertion of an additional guanine increases transcriptional activity due to the creation of a core binding site for a transcription factor from the Ets family (19). It would be interesting to test whether this polymorphism favors tissue destruction and enhances risk of disease progression in TB. Supporting this idea, a recent study reported that the presence of the –1607G allele was significantly higher in TB patients with advanced pulmonary fibrosis compared with that in those with minimal fibrosis (20).

Fibulin-5: two for the price of one maintaining pelvic support

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nisms of POP. Because of the difficulty of procuring human tissue and the expense associated with studying POP in primates (6), rodent models of elastinopathies have been used to study this disease (7–9). While rodents are quadrupeds and, by definition, have different forces operating on the pelvic floor, the connective tissue anatomy is grossly similar to that seen in humans (10). Although pelvic floor injury is recognized as a common antecedent to the development of POP, an improved understanding of the subsequent cellular and genetic alterations in connective tissue is required to improve therapeutic strategies. In this issue of the JCI, Budatha et al. (11) exploit murine genetic models to develop a unifying molecular mechanism of abnormal elastic fiber homeostasis leading to POP and identify fibulin-5 as the central player in the pathophysiology of this disorder (12).

Elastinopathy in pelvic organ prolapse

Because women suffering from connective tissue disorders such as Marfan or Ehlers-Danlos syndromes have high rates of spontaneous pelvic organ prolapse (13), considerable interest has been directed at understanding alterations in connective tissue homeostasis that contribute to the etiology of prolapse. The pelvic floor is supported by an ECM composed of collagen, elastic fibers, and proteoglycans, and several published reports associate POP with changes in these ECM components and in the activity of MMPs (14–16).

MMPs play an important role in tissue remodeling and collectively form a 26-member family of proteases that are mainly responsible for proteolytic degradation of the ECM proteins, including collagens, gelatins, and elastin. Although degradation has been shown to be an important part of MMP function, recent data suggest that MMPs can also affect cellular growth and behavior through the interaction between MMP substrates with ECM-independent factors, including growth factors, cytokines, adhesion receptors, and cell surface proteoglycans (17). Previous studies have identified MMP-2 and -9 as the primary elastolytic enzymes involved in the pathogenesis of POP (3). Utilizing zymography, Jackson et al. demonstrated increased active MMP-2 and -9 in women with POP compared with controls (14). Other groups have demonstrated a similar increase in the active form of MMP-9 in association with POP (3). Interestingly, an increase in MMP-9 activity does not necessarily correspond to an increased transcript level in prolapsed vaginal tissue, suggesting that posttranscriptional regulation of MMP-9 occurs in POP (15). Taken together, these data signify that increased ECM turnover is an essential process in this disorder.

While mature, insoluble elastin is generally stable after development throughout the life span (18), elastic fibers in the female reproductive tract undergo frequent turnover under normal physiologic conditions. Elastin is an insoluble and durable cross-linked array synthesized by joining soluble tropoelastin protein molecules in a reaction catalyzed by lysyl oxidase (LOX) (Figure 1) and provides expansion and recoil in response to tension, which supports the pelvic floor during parturition. Elastic fibers normally degrade during labor and are resynthesized following vaginal delivery (19); this process is under hormonal control and is mediated by age (20). Wieslander et al. (19) demonstrated increased MMP-2 and MMP-9 enzyme activities with a corresponding decrease in protease inhibitors (TIMP2, cystatin C, and α-1 antitrypsin) in genetically normal mice 48 hours after delivery of pups. It is theorized that abnormalities in this normal process of degradation and renewal could lead to POP as women age.

**Murine models of POP**

LOXs are a family of five enzymes that catalyze the cross-linking of collagen and elastin (21). LOX-like 1 protein (LOXL1) appears to be the most important factor in vaginal tissue remodeling that occurs after parturition and in POP associated with aging (20). LOXL1-knockout mice (Loxl1−/−) demonstrate multiple elastinopathies early in life but do not develop spontaneous POP until after 12 weeks of age or delivery of first and second litters (7). Loxl1−/− mice have significantly lower vaginal elastin content and, together with absent normal fluctuations in LOXL1 throughout pregnancy and parturition, this leads to abnormal renewal of the ECM following delivery compared with WT animals (7). These studies suggest that synthesis and degradation of elastic fibers both play a role in vaginal wall remodeling.

Another family of proteins important in elastic fiber matrix assembly and function are fibulins. Fibulins are secreted glycoproteins that become incorporated into a fibrillar extracellular matrix. The seven known members of the fibulin family (fibulins 1–7) have a series of EGF-like domains followed by a C-terminal “fibulin-type” domain (22). Both fibulin-3 and

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**Figure 1**

Model of elastogenesis. N-terminal region of LOXL1 binds to C-terminal domain of fibulin-5 to activate tropoelastin by converting to lysyl-deaminated form. Covalent cross-linking of tropoelastin occurs to form mature elastin. There are additional regulatory processes in elastogenesis that are tissue specific.
fibulin-5 have been implicated as having a role in maintaining pelvic support (8, 9). Fibulin-3-knockout mice (Fbln3−/−) have abdominal wall fascial defects and variably develop POP that increases with age. More-over, the Fbln3−/− animals that exhibit POP also have increased levels of active MMP-2 and MMP-9 compared with Fbln3−/− animals without prolapse or WT animals (9). However, these animals experience normal tissue recovery following parturition. This latter finding could be related to the observed compensatory increase in mRNA transcripts for protease inhibitors (TIMP1, TIMP3), fibulin-5, and tropoelastin (9).

Fibulin-5–knockout mice (Fbln5−/−) consistently develop prolapse with advancing age: 91% of Fbln5−/− animals develop POP by 6 months of age, while this is true of only 50% and 27% of older Lox1−/− and Fbln3−/− animals, respectively (7–9). Additionally, increased mononuclear infiltration in the vaginal wall during labor seen in Fbln5−/− animals may indicate increased elastolytic activity, further suggesting that both abnormal synthesis and increased degradation are necessary to develop POP (8). Given the aforementioned findings in both Fbln5−/− and Lox1−/− animals, Budatha et al. sought to further explain the interactions between fibulin-5 and LOXL1 in the pathogenesis of POP (11).

Fibulin-5 promotes cell adhesion by interacting with integrins via its RGD motif. Budatha et al. generated mice homozygous for alleles of Fbln5 in which the RGD motif was mutated (Fbln5RGE) and found that these mice had elastic fibers and ECM protein content that was comparable to that of WT animals (11). In contrast to the Fbln5−/− animals, POP was not observed in the Fbln5RGE/RGE animals after pregnancy or with advancing age, suggesting that integrin-binding activity of fibulin-5 does not affect normal elastic fiber formation (Figure 2). The authors further demonstrated a significant increase in MMP-9 activity in vaginal tissues from the Fbln5RGE/RGE and Fbln5−/− animals compared with WT. They determined that fibulin 5 indirectly mediates the upregulation of MMP-9 in an RGD-dependent manner. ROS also upregulate MMP-9 in vaginal stromal cells from both fibulin mutant mouse models, suggesting that the fibulin-5 integrin interaction is a mediator in the pathway between ROS and MMP-9 activity in the vaginal wall (Figure 2).

What is most interesting about this study is that, although Fbln5RGE/RGE mice do not appear to develop POP or abnormal elastic fiber assembly, they do develop a milder form of disease when LOX activity is blocked by treating animals with β-aminopropionitrile (BAPN). If this is due to impaired de novo elastin synthesis, as the authors suggest, it would be interesting to see whether more severe POP develops in animals treated with BAPN for longer periods of time. To further demonstrate the necessity of both MMP-9 activity and abnormal elastic fiber synthesis for POP, Budatha et al. generated mice that were deficient in both MMP-9 and fibulin-5 to form a double KO (DKO) model (11). Only 42% of the DKO mice developed POP,
while 95% of the Fbn5−/− animals developed the disorder, strengthening the argument that MMP-9 upregulation accounts for a significant part of the pelvic floor dysfunction in Fbn5−/− mice.

Concluding remarks
There is increasing evidence that POP results from alterations in the relative balance between synthesis and degradation of the ECM in pelvic tissues. In this paper, Budatha et al. have elegantly established a dual role for fibrillin-5 in the regulation of elastic fiber homeostasis (11). They demonstrate that fibrillin-5 not only facilitates the normal assembly of elastic fibers, but also inhibits MMP-9 through RGID-dependent integrin-binding. In addition, they provide evidence that both abnormal elastic fibers and upregulation of MMP-9 are necessary and sufficient for the full manifestation of POP in murine models.

Although these results have generated important information about the elastinopathic pathways involved in POP, several questions remain. It is still not known how hormonal and aging mechanisms have an impact on these pathways. This is highlighted by the human studies included in this paper. The researchers demonstrated that although MMP-9 was significantly increased in women with prolapse compared with controls, the difference among postmenopausal women (prolapse vs. control) was a large 23-fold change while, among the premenopausal women, there was only a modest 3-fold change. These results, and those of other groups (3), suggest that hormonal modulation is an important factor and should be considered in all molecular pathways that lead to POP.

Other human studies have suggested that alterations in genes responsible for elastic fiber homeostasis are more common in women with clinical manifestations of POP (23), and family history is a known risk factor for the development of this disease (24). It would be important to understand how genetic susceptibility plays into the elastinopathic pathways identified in murine models. Further research is needed to phenotype individuals to determine who is at risk for developing POP primarily or to identify those at risk of recurrence after standard therapies.

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