Oxytalan fibers are a complex structure composed of microfibrillar and microfibril-associated proteins, and have been suggested to play an important role in elasticity and flexibility of the ligament tissues such as periodontal ligaments (PDL). However, little is known about the molecular mechanisms of oxytalan fiber formation. Here we report the novel extracellular protein ADAMTSL-4 is involved in oxytalan fiber formation. Immunohistochemical analysis showed that ADAMTSL4 was initially expressed in dental follicle cells at the PDL forming-stage of tooth germ, and became assembled as oxytalan fibers in the adult PDL. Overexpression of ADAMTSL4 in MG63 cells induced formation of oxytalan fiber-like microfibril assembly. To investigate the functional role of ADAMTSL4, we examined whether ADAMTSL4 associated with fibrillin-1, a major microfibril component of oxytalan fiber. Double immunostaining analysis showed that ADAMTSL4 was colocalized with fibrillin-1 in cells overexpressing ADAMTSL4 and in the adult PDL. Mice homozygous for hypomorphic allele (MgR) of fibrillin-1 showed abnormal oxytalan fiber assembly of ADAMTSL4, and significant disorganization of PDL. Our results suggest that ADAMTSL4 associates with fibrillin-1 during the assembly of oxytalan fibers, and that these complexes play an important role in the formation of PDL.