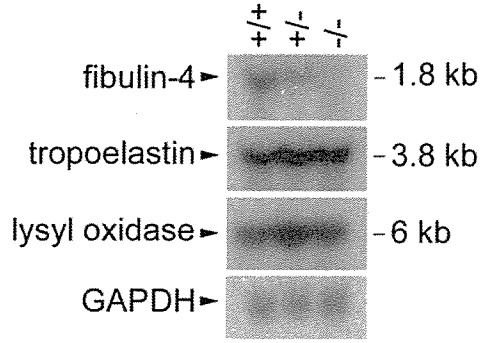

FIG. 7. Unaffected tropoelastin and lysyl oxidase expression in *fibulin-4*$^{-/-}$ mice. Northern blot analysis of equal amount of mouse lung total RNA shows a *fibulin-4* mRNA transcript of 1.8 kb detectable in wild-type (+/+) and heterozygous (+/−) but not homozygous (−/−) mice (top). The intensity of the signal is reduced in heterozygous mice. The same blot stripped and reblotted with a tropoelastin probe shows a 3.8-kb tropoelastin transcript with similar intensity in all the mice (second panel from the top). Probing with a *Lox* probe indicates a 6-kb *Lox* transcript with similar levels in all mice (third panel). A GAPDH probe was used as a control (bottom).

fected into 293T cells. Tropoelastin was expressed at a very low level. We could detect tropoelastin in the cell lysate, but it was difficult to detect it in the culture medium, presumably due to its self-coacervation and formation of insoluble elastin in the medium. Fibulin-4 was expressed at a relatively high level and could be readily detected in both lysate and medium. Thus, we chose to use transfected cell lysates for the coimmunoprecipitation assay. As shown in Fig. 8C, tropoelastin was immunoprecipitated from lysates transfected with either tropoelastin alone (lane 2) or both tropoelastin and fibulin-4 (lane 1) by the antitropoelastin antibody. It was also coimmunoprecipitated by an anti-fibulin-4 monoclonal antibody from the lysate cotransfected with tropoelastin and fibulin-4 (lane 3). The coimmunoprecipitation did not appear to be due to a nonspecific association of tropoelastin with the fibulin-4 antibody beads, as no tropoelastin signal was detected from the immunoprecipitate by the same antibody from the lysate transfected only with tropoelastin (lane 4). Reciprocally, fibulin-4 was coimmunoprecipitated by the anti-tropoelastin antibody from the lysate transfected with both tropoelastin and fibulin-4 (lane 7) but not from the lysate transfected with fibulin-4 alone (lane 8), while it was immunoprecipitated by the fibulin-4 antibody from both lysates (lanes 5 and 6). These results demonstrate that fibulin-4 binds specifically with tropoelastin.

To further determine whether exogenous fibulin-4 is colocalized to elastic fibers, we added FLAG-tagged recombinant fibulin-4 to cultured human fibroblasts. These cells are capable of developing a network of elastic fibers in vitro. Double labeling of antitropoelastin and anti-FLAG antibodies showed colocalization of fibulin-4 and elastin (Fig. 9). These data indicate that fibulin-4 assembles into elastic fibers.

DISCUSSION

Elastic fiber formation is thought to involve extrinsic proteins and an intrinsic capacity for elastin coacervation. How extrinsic proteins cooperate with each other and with elastin coacervation to form functional elastic fibers is unknown. Over 30 molecules have been reported to associate with elastic fibers in morphological studies and in vitro assays (23). But many

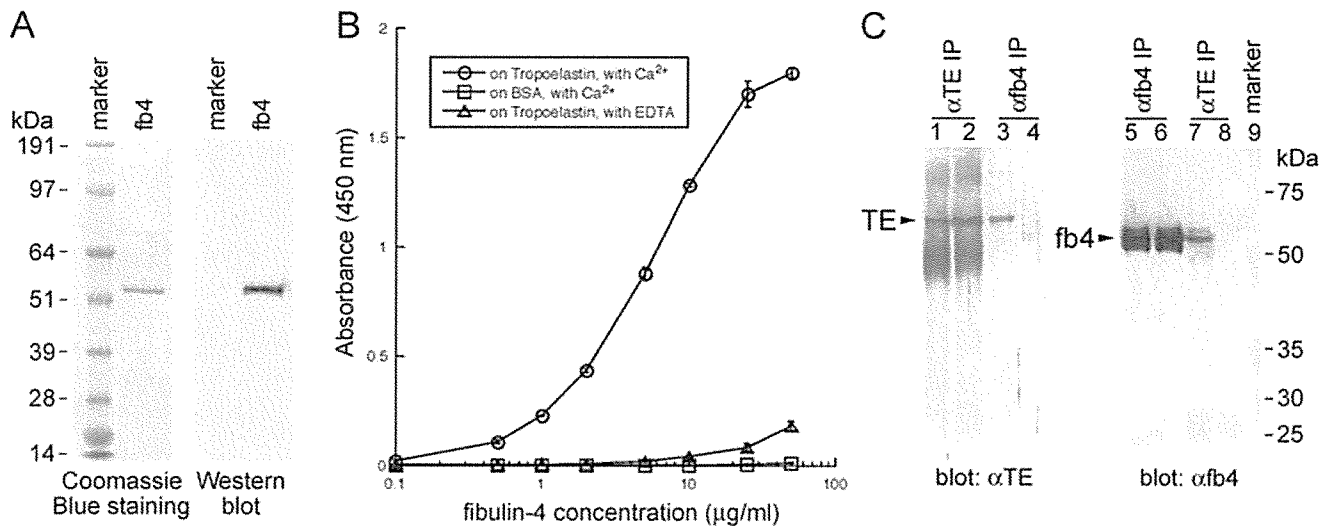

FIG. 8. Fibulin-4 interacts with tropoelastin. (A) Purified recombinant mouse fibulin-4. A total of 1 μg of purified fibulin-4 was used for the Coomassie blue-stained gel, and 10 ng of the protein was used for the Western blot. An anti-FLAG antibody was used for immunodetection. A single band with the correct mass for fibulin-4 was detected by both Coomassie blue staining and immunoblotting. (B) Solid-phase binding assay using recombinant fibulin-4 as a soluble ligand. Note that fibulin-4 binds to tropoelastin in the presence of Ca^{2+} but that the binding is inhibited in the presence of EDTA. Data were obtained as the results of triplicate experiments, and values shown are means ± standard deviations. (C) Coimmunoprecipitation of fibulin-4 with tropoelastin. Lanes 1 to 4, antitropoelastin blotting of immunoprecipitates from lysates of 293T cells transfected with both tropoelastin and fibulin-4 (lanes 1 and 3) or tropoelastin alone (lanes 2 and 4). Tropoelastin was detected in the immunoprecipitate by anti-fibulin-4 (7B9) (lane 3). Lanes 5 to 8, anti-fibulin-4 (11E2) blotting of immunoprecipitates from lysates of 293T cells transfected with both tropoelastin and fibulin-4 (lanes 5 and 7) or fibulin-4 alone (lanes 6 and 8). Fibulin-4 was detected in the immunoprecipitate by antitropoelastin (lane 7). IP, immunoprecipitation; TE, tropoelastin; fb4, fibulin-4.

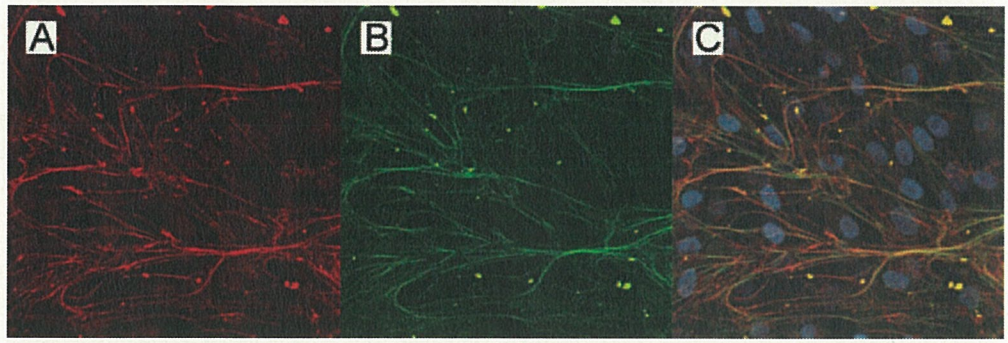

FIG. 9. Colocalization of fibulin-4 and elastin. (A to C) Normal human skin fibroblasts were cultured with FLAG-tagged recombinant fibulin-4 protein. (A) Cells stained with antitropoelastin antibody, showing a network structure. (B) Cells stained with anti-FLAG antibody, also showing a network structure. (C) Superimposed image of panels A and B, with DAPI nuclear staining showing fibulin-4 localization on elastic fibers.

have been found to have no effect or marginal effects on elastic fiber formation in vivo. In this study, we demonstrated that mice lacking fibulin-4 do not form elastic fibers. This is the first report that lack of a protein other than elastin itself completely abolishes the formation of elastic fibers in vivo, indicating that fibulin-4 plays an indispensable role in elastogenesis.

The initial abnormality present in *fibulin-4*$^{-/-}$ mice, arterial narrowing, is similar to that observed with *ELN*$^{-/-}$ mice (27). In *ELN*$^{-/-}$ mice, however, the change is caused by subendothelial cell overproliferation due to the lack of elastin, a process that eventually obliterates the vascular lumen (27). In *fibulin-4*$^{-/-}$ mice, tropoelastin mRNA levels are similar to those in wild-type mice, there is no sign of cell overproliferation and vascular occlusion, and irregular elastin aggregates accumulate with age, suggesting that fibulin-4 does not affect the synthesis of tropoelastin. The formation of functional elastic fibers requires the deposition of tropoelastin at the fiber assembly site, cross-linking of tropoelastin monomers by lysyl oxidase family enzymes, and the organization of resulting insoluble elastin matrix into mature fibers. Fibulin-4 likely affects one or more of these processes.

The irregular elastin aggregates observed in *fibulin-4*$^{-/-}$ mice are highly unusual in that they contain electron-dense rod-like filaments. These filaments are evenly distributed in the aggregates in *fibulin-4*$^{-/-}$ mice, as if, without fibulin-4, a molecule(s) associated with tropoelastin is incorporated together with each tropoelastin monomer into elastin aggregates. Alternatively, the rod-like filament may be a regular elastic fiber component whose presence is revealed by the absence of fibulin-4. The morphology of *fibulin-4*$^{-/-}$ elastin aggregates is similar to that of abnormal elastin aggregates permeated by proteoglycans in the presence of lysyl oxidase inhibitors (13). Also, ECM proteoglycans containing sulfated glycosaminoglycans visualized by a cationic copper phthalocyanin dye, cupromeronic blue, exhibit morphology very similar to the rod-like filaments observed in irregular elastin aggregates in *fibulin-4*$^{-/-}$ mice (48). These observations suggest that the filaments in *fibulin-4*$^{-/-}$ elastin aggregates may be proteoglycans. Glycosaminoglycans containing sulfate groups (chondroitin, dermatan, and heparan sulfate) and their associated proteoglycans have been shown to directly interact with tropoelastin and are normal components of elastic fibers (3, 7, 17, 55). Both chondroitin sulfate and heparan sulfate can mediate tropoelastin's coacervation (17, 24, 45, 53, 55). Decreased elastin deposition was observed when the matrix was depleted of sulfated molecules by chlorate treatment of the cells (8, 53). We also found that fibulin-4 interacts with tropoelastin directly and assembles into elastic fibers in culture. Thus, fibulin-4 may play a role in the initial deposition of tropoelastin, such as scaffolding and facilitating the formation of homogeneous elastin polymers by preventing the association of other molecules with tropoelastin or coordinating tropoelastin and other elastic fiber components during elastic fiber assembly. The identity of the rod-like filaments, the precise roles of proteoglycans in elastogenesis, and their relationships with fibulin-4 remain to be determined.

Elastin cross-linking is severely affected in *fibulin-4*$^{-/-}$ mice. Desmosine was reduced by over 85% in *fibulin-4*$^{-/-}$ mice, compared to levels in wild-type mice. Interestingly, there was a near-20% increase in desmosine in *fibulin-4*$^{+/-}$ mice compared to wild-type mice, although this difference was not statistically significant with the number of animals we analyzed. It is possible that there is more cross-linking in heterozygous mutants than in wild-type mice to compensate for fibulin-4 haploinsufficiency. Elastin cross-linking is catalyzed by lysyl oxidases, a family of enzymes that catalyzes the oxidative deamination of lysine residues in elastin and collagen (21). Five members have been described so far (12, 30). LOX has been shown to be necessary for elastic fiber and collagen fiber development (20, 31), and LOX-like 1 (LOXL1) is required in elastic fiber homeostasis (28). Similar to *fibulin-4*$^{-/-}$ mice, *Lox*$^{-/-}$ mice exhibit severe vascular defects and die perinatally (20, 31). The vascular defects include artery tortuosity, irregularity, and ruptured aneurysms with fragmented elastic lamina in the aortic walls. Desmosine content is decreased by 60%; hydroxyproline, which represents collagen content, is decreased by 30% in *Lox*$^{-/-}$ mice (20). The similarities in gross defects between *fibulin-4*$^{-/-}$ mice and *Lox*$^{-/-}$ mice and loss of desmosine content in *fibulin-4*$^{-/-}$ mice suggest that lack of fibulin-4 may affect the function of lysyl oxidase. However, *Lox* mRNA expression is similar in wild-type and *fibulin-4*$^{-/-}$ mice, hydroxyproline content is not altered in *fibulin-4*$^{-/-}$ mice, and no unusual elastic fiber content such as rod-like filaments found in *fibulin-4*$^{-/-}$ mice has been reported with *Lox*$^{-/-}$ mice. Loxl1-null mice survive to adulthood but do not deposit normal elastic fibers in the uterine tract postpartum; they develop

pelvic prolapse, enlarged airspaces of the lung, loose skin, and vascular abnormalities. Desmosine content in *Loxl1*$^{-/-}$ mice is reduced by 30 to 50%, depending on tissues (28). The difference in elastic fiber defects in *fibulin-4*$^{-/-}$, *Lox*$^{-/-}$, and *Loxl1*$^{-/-}$ mice suggests that fibulin-4 has different roles in elastic fiber assembly, even if it also affects the activities of lysyl oxidases.

Fibulin-4 shares high homology with fibulin-5, with a similar domain structure and >50% amino acid identity. Fibulin-5-null mice grow to adulthood but exhibit loose skin, lung airspace enlargement, and a stiff and tortuous aorta, due to disorganized and fragmented elastic fibers (40, 56). It has been proposed that fibulin-5 may be involved in elastogenesis by tethering elastic fibers onto cell surface integrins and by affecting cross-linking of elastin through direct binding with LOXL1 (28, 40, 56). *Loxl1*$^{-/-}$ mice exhibit similar but less-severe elastic fiber defects than *fibulin-5*$^{-/-}$ mice. Despite the high homology between fibulin-4 and -5, the *fibulin-4*$^{-/-}$ phenotype is not compensated for by fibulin-5. *fibulin-4*$^{-/-}$ mice exhibited almost complete loss of elastic fibers and perinatal lethality, suggesting a more essential role of fibulin-4 in elastogenesis than that of fibulin-5.

With age and some pathological conditions, elastic fibers exhibit interwoven filaments (41) that are similar to the morphology of elastin aggregates of *fibulin-4*$^{-/-}$ mice. Thus, alteration of the function or structure of fibulin-4 may be a major mechanism behind aging and elastic fiber-related diseases. *fibulin-4*$^{-/-}$ mice exhibit the most severe elastinopathy described to date. Understanding the role of fibulin-4 is a prerequisite for understanding the mechanism responsible for elastogenesis. In addition to the crucial role in elastogenesis, fibulin-4 may also have other important functions in cell proliferation and differentiation. Several studies have found that fibulin-4 stimulates cell growth and is upregulated in tumors (14, 16, 19). *fibulin-4*$^{-/-}$ mice will also be a valuable model in further studying these functions.

ACKNOWLEDGMENTS

We thank Peggy McCuskey for electron microscopy and Anna Yocom for technical assistance.

This work was supported by NIH/NEI grants EY13847 and EY13160, Research to Prevent Blindness, Philip Morris USA and Philip Morris International, Health and Labor Sciences research grants, Japan Society for the Promotion of Science, and Japan Science and Technology Agency.

REFERENCES

1. **Argraves, W. S., L. M. Greene, M. A. Cooley, and W. M. Gallagher.** 2003. Fibulins: physiological and disease perspectives. EMBO Rep. **4:**1127–1131.
2. **Arteaga-Solis, E., B. Gayraud, S. Y. Lee, L. Shum, L. Sakai, and F. Ramirez.** 2001. Regulation of limb patterning by extracellular microfibrils. J. Cell Biol. **154:**275–281.
3. **Baccarani-Contri, M., D. Vincenzi, F. Cicchetti, G. Mori, and I. Pasquali-Ronchetti.** 1990. Immunocytochemical localization of proteoglycans within normal elastin fibers. Eur. J. Cell Biol. **53:**305–312.
4. **Bressan, G. M., I. Castellani, M. G. Giro, D. Volpin, C. Fornieri, and I. Pasquali Ronchetti.** 1983. Banded fibers in tropoelastin coacervates at physiological temperatures. J. Ultrastruct. Res. **82:**335–340.
5. **Bressan, G. M., I. Pasquali-Ronchetti, C. Fornieri, F. Mattioli, I. Castellani, and D. Volpin.** 1986. Relevance of aggregation properties of tropoelastin to the assembly and structure of elastic fibers. J. Ultrastruct. Mol. Struct. Res. **94:**209–216.
6. **Bressler, N. M., S. B. Bressler, and S. L. Fine.** 1988. Age-related macular degeneration. Surv. Ophthalmol. **32:**375–413.
7. **Broekelmann, T. J., B. A. Kozel, H. Ishibashi, C. C. Werneck, F. W. Keeley, L. Zhang, and R. P. Mecham.** 2005. Tropoelastin interacts with cell-surface glycosaminoglycans via its COOH-terminal domain. J. Biol. Chem. **280:**40939–40947.
8. **Buczek-Thomas, J. A., C. L. Chu, C. B. Rich, P. J. Stone, J. A. Foster, and M. A. Nugent.** 2002. Heparan sulfate depletion within pulmonary fibroblasts: implications for elastogenesis and repair. J. Cell Physiol. **192:**294–303.
9. **Chaudhry, S. S., J. Gazzard, C. Baldock, J. Dixon, M. J. Rock, G. C. Skinner, K. P. Steel, C. M. Kielty, and M. J. Dixon.** 2001. Mutation of the gene encoding fibrillin-2 results in syndactyly in mice. Hum. Mol. Genet. **10:**835–843.
10. **Chu, M. L., and T. Tsuda.** 2004. Fibulins in development and heritable disease. Birth Defects Res. C Embryo Today **72:**25–36.
11. **Cox, B. A., B. C. Starcher, and D. W. Urry.** 1974. Communication: coacervation of tropoelastin results in fiber formation. J. Biol. Chem. **249:**997–998.
12. **Csiszar, K.** 2001. Lysyl oxidases: a novel multifunctional amine oxidase family. Prog. Nucleic Acid Res. Mol. Biol. **70:**1–32.
13. **Fornieri, C., M. Baccarani-Contri, D. Quaglino, Jr., and I. Pasquali-Ronchetti.** 1987. Lysyl oxidase activity and elastin/glycosaminoglycan interactions in growing chick and rat aortas. J. Cell Biol. **105:**1463–1469.
14. **Gallagher, W. M., M. Argentini, V. Sierra, L. Bracco, L. Debussche, and E. Conseiller.** 1999. MBP1: a novel mutant p53-specific protein partner with oncogenic properties. Oncogene **18:**3608–3616.
15. **Gallagher, W. M., C. A. Currid, and L. C. Whelan.** 2005. Fibulins and cancer: friend or foe? Trends Mol. Med. **11:**336–340.
16. **Gallagher, W. M., L. M. Greene, M. P. Ryan, V. Sierra, A. Berger, P. Laurent-Puig, and E. Conseiller.** 2001. Human fibulin-4: analysis of its biosynthetic processing and mRNA expression in normal and tumour tissues. FEBS Lett. **489:**59–66.
17. **Gheduzzi, D., D. Guerra, B. Bochicchio, A. Pepe, A. M. Tamburro, D. Quaglino, S. Mithieux, A. S. Weiss, and I. Pasquali Ronchetti.** 2005. Heparan sulphate interacts with tropoelastin, with some tropoelastin peptides and is present in human dermis elastic fibers. Matrix Biol. **24:**15–25.
18. **Giltay, R., R. Timpl, and G. Kostka.** 1999. Sequence, recombinant expression and tissue localization of two novel extracellular matrix proteins, fibulin-3 and fibulin-4. Matrix Biol. **18:**469–480.
19. **Heine, H., R. L. Delude, B. G. Monks, T. Espevik, and D. T. Golenbock.** 1999. Bacterial lipopolysaccharide induces expression of the stress response genes hop and H411. J. Biol. Chem. **274:**21049–21055.
20. **Hornstra, I. K., S. Birge, B. Starcher, A. J. Bailey, R. P. Mecham, and S. D. Shapiro.** 2003. Lysyl oxidase is required for vascular and diaphragmatic development in mice. J. Biol. Chem. **278:**14387–14393.
21. **Kagan, H. M., and P. C. Trackman.** 1991. Properties and function of lysyl oxidase. Am. J. Respir. Cell Mol. Biol. **5:**206–210.
22. **Kaufman, M. H., and J. B. L. Bard.** 1999. The Anatomical basis of mouse development. Academic Press, London, United Kingdom.
23. **Kielty, C. M., M. J. Sherratt, and C. A. Shuttleworth.** 2002. Elastic fibres. J. Cell Sci. **115:**2817–2828.
24. **Kielty, C. M., S. P. Whittaker, and C. A. Shuttleworth.** 1996. Fibrillin: evidence that chondroitin sulphate proteoglycans are components of microfibrils and associate with newly synthesised monomers. FEBS Lett. **386:**169–173.
25. **Kostka, G., R. Giltay, W. Bloch, K. Addicks, R. Timpl, R. Fassler, and M. L. Chu.** 2001. Perinatal lethality and endothelial cell abnormalities in several vessel compartments of fibulin-1-deficient mice. Mol. Cell. Biol. **21:**7025–7034.
26. **Kozel, B. A., H. Wachi, E. C. Davis, and R. P. Mecham.** 2003. Domains in tropoelastin that mediate elastin deposition in vitro and in vivo. J. Biol. Chem. **278:**18491–18498.
27. **Li, D. Y., B. Brooke, E. C. Davis, R. P. Mecham, L. K. Sorensen, B. B. Boak, E. Eichwald, and M. T. Keating.** 1998. Elastin is an essential determinant of arterial morphogenesis. Nature **393:**276–280.
28. **Liu, X., Y. Zhao, J. Gao, B. Pawlyk, B. Starcher, J. A. Spencer, H. Yanagisawa, J. Zuo, and T. Li.** 2004. Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. Nat. Genet. **36:**178–182.
29. **Loeys, B., L. Van Maldergem, G. Mortier, P. Coucke, S. Gerniers, J. M. Naeyaert, and A. De Paepe.** 2002. Homozygosity for a missense mutation in fibulin-5 (FBLN5) results in a severe form of cutis laxa. Hum. Mol. Genet. **11:**2113–2118.
30. **Maki, J. M., and K. I. Kivirikko.** 2001. Cloning and characterization of a fourth human lysyl oxidase isoenzyme. Biochem. J. **355:**381–387.
31. **Maki, J. M., J. Rasanen, H. Tikkanen, R. Sormunen, K. Makikallio, K. I. Kivirikko, and R. Soininen.** 2002. Inactivation of the lysyl oxidase gene Lox leads to aortic aneurysms, cardiovascular dysfunction, and perinatal death in mice. Circulation **106:**2503–2509.
32. **Markova, D., Y. Zou, F. Ringpfeil, T. Sasaki, G. Kostka, R. Timpl, J. Uitto, and M. L. Chu.** 2003. Genetic heterogeneity of cutis laxa: a heterozygous tandem duplication within the fibulin-5 (FBLN5) gene. Am. J. Hum. Genet. **72:**998–1004.
33. **Marmorstein, A. D., L. Y. Marmorstein, M. Rayborn, X. Wang, J. G. Hollyfield, and K. Petrukhin.** 2000. Bestrophin, the product of the Best vitelliform macular dystrophy gene (*VMD2*), localizes to the basolateral plasma membrane of the retinal pigment epithelium. Proc. Natl. Acad. Sci. USA **97:**12758–12763.

34. **Marmorstein, L. Y., A. V. Kinev, G. K. Chan, D. A. Bochar, H. Beniya, J. A. Epstein, T. J. Yen, and R. Shiekhattar.** 2001. A human BRCA2 complex containing a structural DNA binding component influences cell cycle progression. Cell **104**:247–257.
35. **Marmorstein, L. Y., P. J. McLaughlin, J. B. Stanton, L. Yan, J. W. Crabb, and A. D. Marmorstein.** 2002. Bestrophin interacts physically and functionally with protein phosphatase 2A. J. Biol. Chem. **277**:30591–30597.
36. **Marmorstein, L. Y., F. L. Munier, Y. Arsenijevic, D. F. Schorderet, P. J. McLaughlin, D. Chung, E. Traboulsi, and A. D. Marmorstein.** 2002. Aberrant accumulation of EFEMP1 underlies drusen formation in malattia leventinese and age-related macular degeneration. Proc. Natl. Acad. Sci. USA **99**:13067–13072.
37. **Mecham, R. P., and E. Davis.** 1994. Elastic fiber structure and assembly, p. 281–314. *In* P. D. Yurchenco, D. E. Birk, and R. P. Mecham (ed.), Extracellular matrix assembly and structure. Academic Press, New York, N.Y.
38. **Midwood, K. S., and J. E. Schwarzbauer.** 2002. Elastic fibers: building bridges between cells and their matrix. Curr. Biol. **12**:R279–R281.
39. **Milewicz, D. M., Z. Urban, and C. Boyd.** 2000. Genetic disorders of the elastic fiber system. Matrix Biol. **19**:471–480.
40. **Nakamura, T., P. R. Lozano, Y. Ikeda, Y. Iwanaga, A. Hinek, S. Minamisawa, C. F. Cheng, K. Kobuke, N. Dalton, Y. Takada, K. Tashiro, J. Ross, Jr., T. Honjo, and K. R. Chien.** 2002. Fibulin-5/DANCE is essential for elastogenesis in vivo. Nature **415**:171–175.
41. **Pasquali-Ronchetti, I., and M. Baccarani-Contri.** 1997. Elastic fiber during development and aging. Microsc. Res. Tech. **38**:428–435.
42. **Pereira, L., K. Andrikopoulos, J. Tian, S. Y. Lee, D. R. Keene, R. Ono, D. P. Reinhardt, L. Y. Sakai, N. J. Biery, T. Bunton, H. C. Dietz, and F. Ramirez.** 1997. Targetting of the gene encoding fibrillin-1 recapitulates the vascular aspect of Marfan syndrome. Nat. Genet. **17**:218–222.
43. **Pereira, L., S. Y. Lee, B. Gayraud, K. Andrikopoulos, S. D. Shapiro, T. Bunton, N. J. Biery, H. C. Dietz, L. Y. Sakai, and F. Ramirez.** 1999. Pathogenetic sequence for aneurysm revealed in mice underexpressing fibrillin-1. Proc. Natl. Acad. Sci. USA **96**:3819–3823.
44. **Pierce, R. A., T. J. Mariani, and R. M. Senior.** 1995. Elastin in lung development and disease. Ciba Found. Symp. **192**:199–212.
45. **Reinboth, B., E. Hanssen, E. G. Cleary, and M. A. Gibson.** 2002. Molecular interactions of biglycan and decorin with elastic fiber components: biglycan forms a ternary complex with tropoelastin and microfibril-associated glycoprotein 1. J. Biol. Chem. **277**:3950–3957.
46. **Rosenbloom, J., W. R. Abrams, and R. Mecham.** 1993. Extracellular matrix 4: the elastic fiber. FASEB J. **7**:1208–1218.
47. **Schultz, D. W., M. L. Klein, A. J. Humpert, C. W. Luzier, V. Persun, M. Schain, A. Mahan, C. Runckel, M. Cassera, V. Vittal, T. M. Doyle, T. M. Martin, R. G. Weleber, P. J. Francis, and T. S. Acott.** 2003. Analysis of the ARMD1 locus: evidence that a mutation in HEMICENTIN-1 is associated with age-related macular degeneration in a large family. Hum. Mol. Genet **12**:3315–3323.
48. **Scott, J. E.** 1980. Collagen-proteoglycan interactions. Localization of proteoglycans in tendon by electron microscopy. Biochem. J. **187**:887–891.
49. **Starcher, B., and M. Conrad.** 1995. A role for neutrophil elastase in the progression of solar elastosis. Connect. Tissue Res. **31**:133–140.
50. **Stone, E. M., T. A. Braun, S. R. Russell, M. H. Kuehn, A. J. Lotery, P. A. Moore, C. G. Eastman, T. L. Casavant, and V. C. Sheffield.** 2004. Missense variations in the fibulin 5 gene and age-related macular degeneration. N. Engl. J. Med. **351**:346–353.
51. **Stone, E. M., A. J. Lotery, F. L. Munier, E. Heon, B. Piguet, R. H. Guymer, K. Vandenburgh, P. Cousin, D. Nishimura, R. E. Swiderski, G. Silvestri, D. A. Mackey, G. S. Hageman, A. C. Bird, V. C. Sheffield, and D. F. Schorderet.** 1999. A single EFEMP1 mutation associated with both malattia leventinese and Doyne honeycomb retinal dystrophy. Nat. Genet. **22**:199–202.
52. **Timpl, R., T. Sasaki, G. Kostka, and M. L. Chu.** 2003. Fibulins: a versatile family of extracellular matrix proteins. Nat Rev. Mol. Cell Biol. **4**:479–489.
53. **Trask, B. C., T. M. Trask, T. Broekelmann, and R. P. Mecham.** 2000. The microfibrillar proteins MAGP-1 and fibrillin-1 form a ternary complex with the chondroitin sulfate proteoglycan decorin. Mol. Biol. Cell **11**:1499–1507.
54. **Trask, T. M., B. C. Trask, T. M. Ritty, W. R. Abrams, J. Rosenbloom, and R. P. Mecham.** 2000. Interaction of tropoelastin with the amino-terminal domains of fibrillin-1 and fibrillin-2 suggests a role for the fibrillins in elastic fiber assembly. J. Biol. Chem. **275**:24400–24406.
55. **Wu, W. J., B. Vrhovski, and A. S. Weiss.** 1999. Glycosaminoglycans mediate the coacervation of human tropoelastin through dominant charge interactions involving lysine side chains. J. Biol. Chem. **274**:21719–21724.
56. **Yanagisawa, H., E. C. Davis, B. C. Starcher, T. Ouchi, M. Yanagisawa, J. A. Richardson, and E. N. Olson.** 2002. Fibulin-5 is an elastin-binding protein essential for elastic fibre development in vivo. Nature **415**:168–171.