Elastic fibre formation

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02/11/2019
**Introduction**

Reactome is an open-source, open-access, manually curated, and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

**Literature references**


Reactome database release: 70

This document contains 2 pathways and 7 reactions (see Table of Contents)
Elastic fibre formation

Stable identifier: R-HSA-1566948

Compartments: extracellular region

Elastic fibres (EF) are a major structural constituent of dynamic connective tissues such as large arteries and lung parenchyma, where they provide essential properties of elastic recoil and resilience. EF are composed of a central cross-linked core of elastin, surrounded by a mesh of microfibrils, which are composed largely of fibrillin. In addition to elastin and fibrillin-1, over 30 ancillary proteins are involved in mediating important roles in elastic fibre assembly as well as interactions with the surrounding environment. These include fibulins, elastin microfibril interface located proteins (EMILINs), microfibril-associated glycoproteins (MAGPs) and Latent TGF-beta binding proteins (LTBPs). Fibulin-5 for example, is expressed by vascular smooth muscle cells and plays an essential role in the formation of elastic fibres through mediating interactions between elastin and fibrillin (Yanigasawa et al. 2002, Freeman et al. 2005). In addition, it plays a role in cell adhesion through integrin receptors and has been shown to influence smooth muscle cell proliferation (Yanigasawa et al. 2002, Nakamura et al. 2002). EMILINs are a family of homologous glycoproteins originally identified in extracts of aortas. Found at the elastin-fibrillin interface, early studies showed that antibodies to EMILIN can affect the process of elastic fibre formation (Bressan et al. 1993). EMILIN1 has been shown to bind elastin and fibulin-5 and appears to coordinate their common interaction (Zanetti et al. 2004). MAGPs are found to co-localize with microfibrils. MAGP-1, for example, binds strongly to an N-terminal sequence of fibrillin-1. Other proteins found associated with microfibrils include vitronectin (Dahlback et al. 1990).

Fibrillin is most familiar as a component of elastic fibres but microfibrils with no elastin are found in the ciliary zonules of the eye and invertebrate circulatory systems. The addition of elastin to microfibrils is a vertebrate adaptation to high pulsatile pressures in their closed circulatory systems (Faury et al. 2003). Elastin appears to have emerged after the divergence of jawless vertebrates from other vertebrates (Sage 1982).

Fibrillin-1 is the major structural component of microfibrils. Fibrillin-2 is expressed earlier in develop-
ment than fibrillin-1 and may be important for elastic fiber formation (Zhang et al. 1994). Fibrillin-3 arose as a duplication of fibrillin-2 that did not occur in the rodent lineage. It was first isolated from human brain (Corson et al. 2004).

Fibrillin assembly is not as well defined as elastin assembly. The primary structure of fibrillin is dominated by calcium binding epidermal growth factor like repeats (Kielty et al. 2002). Fibrillin may form dimers or trimers before secretion. However, multimerisation predominantly occurs outside the cell. Formation of fibrils appears to require cell surface structures suggesting an involvement of cell surface receptors. Fibrillin is assembled pericellularly (i.e. on or close to the cell surface) into microfibrillar arrays that undergo time dependent maturation into microfibrils with beaded-string appearance. Transglutaminase forms gamma glutamyl epsilon lysine isopeptide bonds within or between peptide chains. Additionally, intermolecular disulfide bond formation between fibrillins is an important contributor to fibril maturation (Reinhardt et al. 2000).

Models of fibrillin-1 microfibril structure suggest that the N-terminal half of fibrillin-1 is asymmetrically exposed in outer filaments, while the C-terminal half is buried in the interior (Kuo et al. 2007). Fibrillino-pathies include Marfan syndrome, familial ectopia lentis, familial thoracic aneurysm, all due to mutations in the fibrillin-1 gene FBN1, and congenital contractural arachnodactyly which is caused by mutation of FBN2 (Maslen & Glanville 1993, Davis & Summers 2012).

In vivo assembly of fibrillin requires the presence of extracellular fibronectin fibres (Sabatier et al. 2009). Fibrillins have Arg-Gly-Asp (RGD) sequences that interact with integrins (Pfaff et al. 1996, Sakamoto et al. 1996, Bax et al., 2003, Jovanovic et al. 2008) and heparin-binding domains that interact with a cell-surface heparan sulfate proteoglycan (Tiedemann et al. 2001) possibly a syndecan (Ritty et al. 2003). Fibrillins also have a major role in binding and sequestering growth factors such as TGF beta into the ECM (Neptune et al. 2003). Proteoglycans such as versican (Isogai et al. 2002), biglycan, and decorin (Reinboth et al. 2002) can interact with the microfibrils. They confer specific properties including hydration, impact absorption, molecular sieving, regulation of cellular activities, mediation of growth factor association, and release and transport within the extracellular matrix (Buczek-Thomas et al. 2002). In addition, glycosaminoglycans have been shown to interact with tropoelastin through its lysine side chains (Wu et al. 1999), regulating tropoelastin assembly (Tu & Weiss 2008).

Elastin is synthesized as a 70kDa monomer called tropoelastin, a highly hydrophobic protein composed largely of two types of domains that alternate along the polypeptide chain. Hydrophobic domains are rich in glycine, proline, alanine, leucine and valine. These amino acids occur in characteristic short (3-9 amino acids) tandem repeats, with a flexible and highly dynamic structure (Floquet et al. 2004). Unlike collagen, glycine in elastin is not rigorously positioned every 3 residues. However, glycine is distributed frequently throughout all hydrophobic domains of elastin, and displays a strong preference for inter-glycine spacing of 0-3 residues (Rauscher et al. 2006).

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Elastic fibre formation involves the deposition of tropoelastin onto a template of fibrillin rich microfibrils. Recent results suggest that the first step of elastic fiber formation is the organization of small globules of elastin on the cell surface followed by globule aggregation into microfibrils (Kozel et al. 2006). An important contribution to the initial stages assembly is thought to be made by the intrinsic ability of the protein to direct its own polymeric organization in a process termed 'coacervation' (Bressan et al. 1986). This self-assembly process appears to be determined by interactions between hydrophobic domains (Bressan et al. 1986, Vrhovski et al. 1997, Bellingham et al. 2003, Cirulis & Keeley 2010) which result in alignment of the cross-linking domains, allowing the stabilization of elastin through the formation of cross-links generated through the oxidative deamination of lysine residues, catalyzed by members of the lysyl oxidase (LOX) family (Reiser et al. 1992, Mithieux & Weiss 2005). The first step in the cross-linking reaction is the oxidative formation of the delta aldehyde, known as alpha aminoacidipic semialdehyde or
allysine (Partridge 1963). Subsequent reactions that are probably spontaneous lead to the formation of cross-links through dehydrolysinonorleucine and allysine aldol, a trifunctional cross-link dehydromerodesmosine and two tetrafunctional cross-links desmosine and isodesmosine (Lucero & Kagan 2006), which are unique to elastin. These cross-links confer mechanical integrity and high durability. In addition to their role in self-assembly, hydrophobic domains provide elastin with its elastomeric properties, with initial studies suggesting that the elastomeric propereties of elastin are driven through changes in entropic interactions with surrounding water molecules (Hoeve & Flory 1974).

A very specific set of proteases, broadly grouped under the name elastases, is responsible for elastin remodelling (Antonicelli et al. 2007). The matrix metalloproteinases (MMPs) are particularly important in elastin breakdown, with MMP2, 3, 9 and 12 explicitly shown to degrade elastin (Ra & Parks 2007). Nonetheless, elastin typically displays a low turnover rate under normal conditions over a lifetime (Davis 1993).

**Literature references**


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Fibrillin C-terminal processing

**Location:** Elastic fibre formation

**Stable identifier:** R-HSA-2129357

**Type:** transition

**Compartments:** plasma membrane

Extracellular deposition of fibrillin requires removal of the C-terminus, which can be cleaved in vitro by several furin/PACE family convertases (Raghunath et al. 1999, Ritty et al. 1999) in a process that is inhibited by N-glycosylation and calreticulin (Ashworth et al. 1999). Furin (PACE) is a transmembrane protein, synthesized as a 100 kDa protein, which rapidly undergoes autocatalytic cleavage to a 94 kDa protein in the endoplasmic reticulum (ER). The propeptide remains bound as an auto-inhibitor. Propeptide release occurs in the acidic pH of the trans-golgi-network (TGN)/endosomal compartment, activating furin. Though furin is primarily localized to the TGN a proportion of furin molecules are found on the cell surface (Teuchert et al. 1999). Profibrillin-1 processing does not occur in the TGN, where it is bound by two ER-resident molecular chaperones, BiP and GRP94. Instead activation by furin occurs as profibrillin-1 is secreted, or immediately after secretion (Wallis et al. 2003).

**Followed by:** Fibrillin microfibril assembly

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Fibrillin-1 binds integrins

Location: Elastic fibre formation

Stable identifier: R-HSA-2328037

Type: binding

Compartments: plasma membrane

Fibrillin-1 splice variants that include the RGD sequence located in the fourth 8-cysteine domain mediate cell adhesion, binding integrin alphaVbeta3 (Pfaff et al. 1996), alpha5beta1 (Bax et al. 2003) and alphaVbeta6 (Jovanovic et al. 2008). AlphaVbeta3 has the highest affinity for fibrillin-1. Integrin alphaVbeta3 is a high-affinity fibrillin-1 receptor (K(d) approximately 40 nM), whereas integrins alphaVbeta6 and alpha5beta1 show moderate-affinity (K(d) approximately 450 nM) and low-affinity (K(d) >1 microM) binding respectively (Jovanovic et al. 2008).

Preceded by: Fibrillin microfibril assembly

Literature references

Fibrillin microfibril assembly

Location: Elastic fibre formation

Stable identifier: R-HSA-2129362

Type: transition

Compartments: extracellular region

Fibrillin microfibril assembly is a cell regulated process, independent of tropoelastin. Distinct microfibril populations have been identified, suggesting that the cellular environment plays a role in regulating microfibril fate (Kielty et al. 2002). Fibrillin 1 may undergo limited assembly into dimers or trimers in the secretory pathway (Ashworth et al. 1999, Trask et al. 1999) but the formation of large microfibril polymers is extracellular. Microfibrils assemble close to the cell surface in a process that may require cell surface receptors. Fibrillins interact with several integrins (Sakamoto et al. 1996, Jovanovic et al. 2008) suggesting an assembly mechanism with similarities to fibronectin matrix formation. Heparan sulphate proteoglycans (HSPGs) and chondroitin sulfate containing proteoglycans (CSPGs) have also been proposed to have a role in assembly (Tiedemann et al. 2001). Fibrillin polymerization into fibres further requires the formation of disulfide bonds between fibrillins (Reinhardt et al. 2000), initially via calcium-binding epidermal growth factor domains at the C-terminus (Hubmacher et al. 2008), and transglutaminase cross-links (Kielty et al. 2002).

Preceded by: Fibrillin C-terminal processing

Followed by: MFAP2, MFAP5 bind microfibrils, Fibrillin-1 binds integrins

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MFAP2, MFAP5 bind microfibrils

Location: Elastic fibre formation

Stable identifier: R-HSA-2129385

Type: binding

Compartments: extracellular region

Microfibrils are composed largely of fibrillin but also contain covalently-linked microfibril associated glycoproteins MAGP-1 (MFAP2) and MAGP-2 (MFAP5). MFAP2 is a structural component of almost all vertebrate microfibrils (Gibson et al. 1989, Trask et al. 2000, Jensen et al. 2001). It appears to be important for tissue development and/or homeostasis, including regulation of bone remodelling and deposition of tissue fat (Weinbaum et al. 2008). The C-terminal half of MFAP2 is rich in cysteines and contains a matrix-binding domain that facilitates interactions with fibrillin (Weinbaum et al. 2008). The related MFAP5 similarly binds to microfibrils (Gibson et al. 1998) but with a restricted expression profile.

Preceded by: Fibrillin microfibril assembly

Followed by: Tropoelastin associates with microfibrils

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**Tropoelastin forms aggregate globules**

**Location:** Elastic fibre formation

**Stable identifier:** R-HSA-2161293

**Type:** omitted

**Compartments:** extracellular region

The core protein representing ~90% of the mass of elastic fibres is elastin, a highly insoluble protein. It is secreted as soluble protein monomers referred to as tropoelastin, which have alternating hydrophobic and cross linking domains. The self-assembly of tropoelastin into a fibrillar elastin matrix is a multi step process. The first step is the self-association of secreted monomers via hydrophobic domains, in a process known as coacervation. This process concentrates monomers and may align residues in the correct register for subsequent cross linking (Yeo et al. 2011). Under physiological conditions the ~15 nm monomers phase-separate and coalesce into spherical packages 2-6 micrometers in diameter (Clarke et al. 2006, Kozel et al. 2004). This process is represented here by the association of an arbitrary 10 tropoelastin monomers. While they grow, coacervate packages are tethered to the cell surface (Wise & Weiss 2009). The binding interactions between tropoelastin and the cell surface are not fully understood but possible partners include integrins and glycosaminoglycans (Broekelmann et al. 2005). Extracellular fibrillin microfibrils act as a scaffold for the deposition of tropoelastin globules as part of elastic fibre formation (Kozel et al. 2004).

**Followed by:** Tropoelastin associates with microfibrils

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Elastin, the highly insoluble core protein of elastic fibers, is secreted as a soluble protein monomer referred to as tropoelastin. Under physiological conditions the monomers phase separate and coalesce into spherical packages (Clarke et al. 2006), a process known as coacervation. Packages of accumulated elastin are delivered to fibrillin-based fibres in a mechanism that is correlated with cell migration during embryonic development (Czirok et al. 2006). A transglutaminase cross-link between domain 4 of tropoelastin and domain 16 of fibrillin-1 (FBN1) may stabilize initial deposition (Clarke et al. 2005). Elastin is subsequently cross linked by members of the lysyl oxidase family via lysine residues, resulting in mature, insoluble fibres (Sato et al. 2007, Wise & Weiss 2009).

Fibulin-5 (FBLN5) expressed by vascular smooth muscle cells plays an essential role in the formation of elastic fibres, mediating interactions between elastin and fibrillin (Yanigasawa et al. 2002, Freeman et al. 2005). FBLN5 binds tropoelastin but not mature elastin (Zheng et al. 2007), regulating coacervation (Yanigasawa et al. 2009). FBLN5 can bind FBN1 monomers and fibrils (Freeman et al. 2005), but it is not clear whether this is necessary for elastin polymerization. FBLN5 also binds elastin cross-linking enzymes lysyl oxidase like (LOXL)-1, -2, and -4 (Hirai et al. 2007). Overexpression of Fbln5 increases elastin deposition and formation of desmosine cross-links (Nonaka et al. 2009). EMILIN can affect the process of elastic fibre formation (Bressan et al. 1993). It binds elastin and fibulin-5 and appears to coordinate their common interaction (Zanetti et al. 2004).

Preceded by: Tropoelastin forms aggregate globules, MFAP2, MFAP5 bind microfibrils

Followed by: Elastin cross-linking by lysyl oxidase

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Elastin cross-linking by lysyl oxidase

Location: Elastic fibre formation

Stable identifier: R-HSA-2129375

Type: transition

Compartments: extracellular region

Soluble monomers of tropoelastin are cross-linked by the oxidative deamination of lysine residues, catalyzed by lysyl oxidase (LOX). The first step in the cross linking reaction is the oxidative formation of the delta-aldehyde, known as alpha amino adipic semialdehyde or allysine (Partridge 1963). Subsequent spontaneous reactions lead to the formation of cross-links through dehydrolysinonorleucine and allysine aldol, a trifunctional cross-link dehydromerodesmosine and two tetrafunctional cross-links desmosine and isodesmosine (Lucero & Kagan 2006), which are unique to elastin.

Preceded by: Tropoelastin associates with microfibrils

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Proteins found associated with microfibrils include vitronectin (Dahlback et al. 1990), latent transforming growth factor beta-binding proteins (Kielty et al. 2002, Munger & Sheppard 2011), emilin (Bressan et al. 1993, Mongiat et al. 2000), members of the microfibrillar-associated proteins (MFAPs, Gibson et al. 1996), and fibulins (Roark et al. 1995, Yanagisawa et al. 2002). The significance of these interactions is not well understood but may help mediate elastin-fibrillin interactions during elastic fibre assembly.

Proteoglycans such as versican (Isogai et al. 2002), biglycan, and decorin (Reinboth et al. 2002) can interact with the microfibrils. They confer specific properties including hydration, impact absorption, molecular sieving, regulation of cellular activities, mediation of growth factor association, and release and transport within the extracellular matrix (Buczek-Thomas et al. 2002). In addition, glycosaminoglycans have been shown to interact with tropoelastin through its lysine side chains (Wu et al. 1999) regulating tropoelastin assembly (Tu and Weiss, 2008).

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