= Exosome complex =

The exosome complex (or PM / Scl complex , often just called the exosome) is a multi @-@ protein intracellular complex capable of degrading various types of RNA (ribonucleic acid) molecules . Exosome complexes are found in both eukaryotic cells and archaea , while in bacteria a simpler complex called the degradosome carries out similar functions .

The core of the exosome contains a six @-@ membered ring structure to which other proteins are attached . In eukaryotic cells , the exosome complex is present in the cytoplasm , nucleus and especially the nucleolus , although different proteins interact with the exosome complex in these compartments regulating the RNA degradation activity of the complex to substrates specific to these cell compartments . Substrates of the exosome include messenger RNA , ribosomal RNA , and many species of small RNAs . The exosome has an exoribonucleolytic function , meaning it degrades RNA starting at one end (the 3 ? end in this case) , and in eukaryotes also an endoribonucleolytic function , meaning it cleaves RNA at sites within the molecule .

Several proteins in the exosome are the target of autoantibodies in patients with specific autoimmune diseases (especially the PM / Scl overlap syndrome) and some antimetabolic chemotherapies for cancer function by blocking the activity of the exosome . In addition , mutations in exosome component 3 cause pontocerebellar hypoplasia and spinal motor neuron disease .

= = Discovery = =

The exosome was first discovered as an RNase in 1997 in the budding yeast Saccharomyces cerevisiae , an often @-@ used model organism . Not long after , in 1999 , it was realized that the exosome was in fact the yeast equivalent of an already described complex in human cells called the PM / Scl complex , which had been identified as an autoantigen in patients with certain autoimmune diseases years earlier (see below) . Purification of this " PM / Scl complex " allowed the identification of more human exosome proteins and eventually the characterization of all components in the complex . In 2001 , the increasing amount of genome data that had become available allowed the prediction of exosome proteins in archaea , although it would take another 2 years before the first exosome complex from an archaeal organism was purified .

= = Structure = =

= = = Core proteins = = =

The core of the complex has a ring structure consisting of six proteins that all belong to the same class of RNases , the RNase PH @-@ like proteins . In archaea there are two different PH @-@ like proteins (called Rrp41 and Rrp42) , each present three times in an alternating order . Eukaryotic exosome complexes have six different proteins that form the ring structure . Of these six eukaryotic proteins , three resemble the archaeal Rrp41 protein and the other three proteins are more similar to the archaeal Rrp42 protein .

Located on top of this ring are three proteins that have an S1 RNA binding domain (RBD) . Two proteins in addition have a K @-@ homology (KH) domain . In eukaryotes , three different " S1 " proteins are bound to the ring , whereas in archaea either one or two different " S1 " proteins can be part of the exosome (although there are always three S1 subunits attached to the complex) .

This ring structure is very similar to that of the proteins RNase PH and PNPase . In bacteria , the protein RNase PH , which is involved in tRNA processing , forms a hexameric ring consisting of six identical RNase PH proteins . In the case of PNPase , which is a phosphorolytic RNA @-@ degrading protein found in bacteria and the chloroplasts and mitochondria of some eukaryotic organisms , two RNase PH domains , and both an S1 and KH RNA binding domain are part of a single protein , which forms a trimeric complex that adopts a structure almost identical to that of the exosome . Because of this high similarity in both protein domains and structure , these complexes

are thought to be evolutionarily related and have a common ancestor . In bacteria , a separate RNase PH protein exists that is involved in transfer RNA processing , which has been shown to adopt a similar six @-@ membered ring structure , but in this case consisting of 6 identical protein subunits . The RNase PH @-@ like exosome proteins , PNPase and RNase PH all belong to the RNase PH family of RNases and are phosphorolytic exoribonucleases , meaning that they use inorganic phosphate to remove nucleotides from the 3 ' end of RNA molecules .

= = = Associated proteins = = =

Besides these nine core exosome proteins , two other proteins often associate with the complex in eukaryotic organisms . One of these is Rrp44 , a hydrolytic RNase , which belongs to the RNase R family of hydrolytic exoribonucleases (nucleases that use water to cleave the nucleotide bonds) . In addition to being an exoribonucleolytic enzyme , Rrp44 also has endoribonucleolytic activity , which resides in a separate domain of the protein . In yeast , Rrp44 is associated with all exosome complexes and has a crucial role in the activity of the yeast exosome complex . While a human homologue of the protein exists , no evidence was found for a long time that its human homologue was associated with the human exosome complex . In 2010 , however , it was discovered that humans have three Rrp44 homologues and two of these can be associated with the exosome complex . These two proteins most likely degrade different RNA substrates due to their different cellular localization , with one being localized in the cytoplasm (Dis3L1) and the other in the nucleus (Dis3) .

The second common associated protein is called Rrp6 (in yeast) or PM / Scl @-@ 100 (in human) . Like Rrp44 , this protein is a hydrolytic exoribonuclease , but in this case of the RNase D protein family . The protein PM / Scl @-@ 100 is most commonly part of exosome complexes in the nucleus of cells , but can form part of the cytoplasmic exosome complex as well .

= = = Regulatory proteins = = =

Apart from these two tightly bound protein subunits , many proteins interact with the exosome complex in both the cytoplasm and nucleus of cells . These loosely associated proteins may regulate the activity and specificity of the exosome complex . In the cytoplasm , the exosome interacts with AU rich element (ARE) binding proteins (e.g. KRSP and TTP) , which can promote or prevent degradation of mRNAs . The nuclear exosome associates with RNA binding proteins (e.g. MPP6 / Mpp6 and C1D / Rrp47 in humans / yeast) that are required for processing certain substrates .

In addition to single proteins , other protein complexes interact with the exosome . One of those is the cytoplasmic Ski complex , which includes an RNA helicase (Ski2) and is involved in mRNA degradation . In the nucleus , the processing of rRNA and snoRNA by the exosome is mediated by the TRAMP complex , which contains both RNA helicase (Mtr4) and polyadenylation (Trf4) activity .

= = Function = =

= = = Enzymatic function = = =

As stated above , the exosome complex contains many proteins with ribonuclease domains . The exact nature of these ribonuclease domains has changed across evolution from bacterial to archaeal to eukaryotic complexes as various activities have been gained and lost . The exosome is primarily a 3 ' -5 ' exoribonuclease , meaning that it degrades RNA molecules from their 3 ' end . Exoribonucleases contained in exosome complexes are either phosphorolytic (the RNase PH @-@ like proteins) or , in eukaryotes , hydrolytic (the RNase R and RNase D domain proteins) . The phosphorolytic enzymes use inorganic phosphate to cleave the phosphodiester bonds - releasing

nucleotide diphosphates . The hydrolytic enzymes use water to hydrolyse these bonds - releasing nucleotide monophosphates .

In archaea , the Rrp41 subunit of the complex is a phosphorolytic exoribonuclease . Three copies of this protein are present in the ring and are responsible for the activity of the complex . In eukaryotes , none of the RNase PH subunits have retained this catalytic activity , meaning the core ring structure of the human exosome has no enzymatically active protein . Despite this loss of catalytic activity , the structure of the core exosome is highly conserved from archea to humans , suggesting that the complex performs a vital cellular function . In eukaryotes , the absence of the phosphorolytic activity is compensated by the presence of the hydrolytic enzymes , which are responsible for the ribonuclease activity of the exosome in such organisms .

As stated above , the hydrolytic proteins Rrp6 and Rrp44 are associated with the exosome in yeast and in humans , besides Rrp6 , two different proteins , Dis3 and Dis3L1 can be associated at the position of the yeast Rrp44 protein . Although originally the S1 domain proteins were thought to have 3 ' -5 ' hydrolytic exoribonuclease activity as well , the existence of this activity has recently been questioned and these proteins might have just a role in binding substrates prior to their degradation by the complex .

= = = Substrates = = =

The exosome is involved in the degradation and processing of a wide variety of RNA species . In the cytoplasm of cells , it is involved in the turn @-@ over of messenger RNA (mRNA) molecules . The complex can degrade mRNA molecules that have been tagged for degradation because they contain errors , through interactions with proteins from the nonsense mediated decay or non @-@ stop decay pathways . In alternative fashion , mRNAs are degraded as part of their normal turnover . Several proteins that stabilize or destabilize mRNA molecules through binding to AU @-@ rich elements in the 3 ' untranslated region of mRNAs interact with the exosome complex . In the nucleus , the exosome is required for the correct processing of several small nuclear RNA molecules . Finally , the nucleolus is the compartment where the majority of the exosome complexes are found . There it plays a role in the processing of the 5.8S ribosomal RNA (the first identified function of the exosome) and of several small nucleolar RNAs .

Although most cells have other enzymes that can degrade RNA , either from the 3 ' or from the 5 ' end of the RNA , the exosome complex is essential for cell survival . When the expression of exosome proteins is artificially reduced or stopped , for example by RNA interference , growth stops and the cells eventually die . Both the core proteins of the exosome complex , as well as the two main associated proteins , are essential proteins . Bacteria do not have an exosome complex ; however , similar functions are performed by a simpler complex that includes the protein PNPase , called the degradosome .

The exosome is a key complex in cellular RNA quality control . Unlike prokaryotes , eukaryotes possess highly active RNA surveillance systems that recognise unprocessed and mis @-@ processed RNA @-@ protein complexes (such as ribosomes) prior to their exit from the nucleus . It is presumed that this system prevents aberrant complexes from interfering with important cellular processes such as protein synthesis .

In addition to RNA processing , turnover and surveillance activities , the exosome is important for the degradation of so @-@ called cryptic unstable transcripts (CUTs) that are produced from thousands of loci within the yeast genome . The importance of these unstable RNAs and their degradation are still unclear , but similar RNA species have also been detected in human cells .

= = Disease = =

= = = Autoimmunity = = =

The exosome complex is the target of autoantibodies in patients suffering from various autoimmune

diseases . These autoantibodies are mainly found in people that suffer from the PM / Scl overlap syndrome , an autoimmune disease in which patients have symptoms from both scleroderma and either polymyositis or dermatomyositis . Autoantibodies can be detected in the serum of patients by a variety of assays . In the past , the most commonly used methods were double immunodiffusion using calf thymus extracts , immunofluorescence on HEp @-@ 2 cells or immunoprecipitation from human cell extracts . In immunoprecipitation assays with sera from anti @-@ exosome positive sera , a distinctive set of proteins is precipitated . Already years before the exosome complex was identified , this pattern was termed the PM / Scl complex . Immunofluorescence using sera from these patients usually shows a typical staining of the nucleolus of cells , which sparked the suggestion that the antigen recognized by autoantibodies might be important in ribosome synthesis . More recently , recombinant exosome proteins have become available and these have been used to develop line immunoassays (LIAs) and enzyme linked immunosorbent assays (ELISAs) for detecting these antibodies .

In these diseases , antibodies are mainly directed against two of the proteins of the complex , called PM / Scl @-@ 100 (the RNase D like protein) and PM / Scl @-@ 75 (one of the RNase PH like proteins from the ring) and antibodies recognizing these proteins are found in approximately 30 % of patients with the PM / Scl overlap syndrome . Although these two proteins are the main target of the autoantibodies , other exosome subunits and associated proteins (like C1D) can be targeted in these patients . At the current time , the most sensitive way to detect these antibodies is by using a peptide , derived from the PM / Scl @-@ 100 protein , as the antigen in an ELISA , instead of complete proteins . By this method , autoantibodies are found in up to 55 % of patients with the PM / Scl overlap syndrome , but they can also be detected in patients suffering from either scleroderma , polymyositis , or dermatomyositis alone .

As the autobodies are found mainly in patients that have characteristics of several different autoimmune diseases , the clinical symptoms of these patients can vary widely . The symptoms that are seen most often are the typical symptoms of the individual autoimmune diseases and include Raynaud 's phenomenon , arthritis , myositis and scleroderma . Treatment of these patients is symptomatic and is similar to treatment for the individual autoimmune disease , often involving either immunosuppressive or immunomodulating drugs .

= = = Cancer treatment = = =

The exosome has been shown to be inhibited by the antimetabolite fluorouracil , a drug used in the chemotherapy of cancer . It is one of the most successful drugs for treating solid tumors . In yeast cells treated with fluorouracil , defects were found in the processing of ribosomal RNA identical to those seen when the activity of the exosome was blocked by molecular biological strategies . Lack of correct ribosomal RNA processing is lethal to cells , explaining the antimetabolic effect of the drug .

= = = Neurological disorders = = =

Mutations in exosome component 3 cause infantile spinal motor neuron disease , cerebellar atrophy , progressive microcephaly and profound global developmental delay , consistent with pontocerebellar hypoplasia type 1B (PCH1B ; MIM 614678) .

= = List of subunits = =

A In archaea several exosome proteins are present in multiple copies , to form the full core of the exosome complex .

B In humans , two different proteins can be associated in this position . In the cytoplasm of cells , Dis3L1 is associated with the exosome , whereas in the nucleus , Dis3 can bind to the core complex .

C Contributes to the ribonucleolytic activity of the complex .