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RNA protein interactions governing expression of the most abundant protein in human body, type I collagen

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Abstract

Type I collagen is the most abundant protein in human body. The protein turns over slowly and its replacement synthesis is low. However, in wound healing or in pathological fibrosis the cells can increase production of type I collagen several hundred fold. This increase is predominantly due to posttranscriptional regulation, including increased half life of collagen mRNAs and their increased translatability. Type I collagen is composed of two α1 and one α2 polypeptides that fold into a triple helix. This stochiometry is strictly regulated to prevent detrimental synthesis of α1 homotrimers. Collagen polypeptides are co-translationally modified and the rate of modifications is in dynamic equilibrium with the rate of folding, suggesting coordinated translation of collagen α1(I) and α2(I) polypeptides. Collagen α1(I) mRNA has in the 3' UTR a C-rich sequence that binds protein αCP, this binding stabilizes the mRNA in collagen producing cells. In the 5'UTR both collagen mRNAs have a conserved stem-loop structure (5'SL). The 5'SL is critical for high collagen expression, knock in mice with disruption of the 5'SL are resistant to liver fibrosis. the 5'SL binds protein LARP6 with strict sequence specificity and high affinity. LARP6 recruits RNA helicase A to facilitate translation initiation and associates collagen mRNAs with vimentin and nonmuscle myosin filaments. Binding to vimentin stabilizes collagen mRNAs, while nonmuscle myosin regulates coordinated translation of α1(I) and α2(I) mRNAs. When nonmuscle myosin filaments are disrupted the cells secrete only α1 homotrimers. Thus, the mechanism governing high collagen expression involves two RNA binding proteins and development of cytoskeletal filaments.

Type I collagen is the most abundant protein in human body. It is a major component of the extracellular matrix of skin, bones, tendons, muscle and tunica adventitia (the outermost layer) of arteries, providing the mechanical strength to these organs[1]. In parenchymal organs it is expressed at low levels to support their architecture. In all organs type I collagen is composed of two α1(I) polypeptides and one α2(I) polypeptide, which fold into a triple helical molecule. Only in the skin homotrimers of α1(I) polypeptides can accumulate up to 10%[2]. Biosynthesis of type I collagen starts with transcription of collagen α1(I) and α2(I) genes and the corresponding mRNAs are translated at the membrane of the endoplasmic reticulum (ER). The nascent polypeptides are co-translationally inserted into the lumen of the ER. During this process they undergo hydroxylations of selected prolines and lysines and glycosylation of selected hydroxy-lysines. Concomitant with these modifications, the polypeptides initiate folding into a triple helix by registration and disulfide bonding of the carboxy terminal ends of two α1(I) polypeptides and one α2(I) polypeptide[3]. Once the C-terminal ends of all three polypeptides are bonded, the folding of the heterotrimeric molecule proceeds in a zipper-like formation towards the N-terminal end. This results in formation of a molecule with the globular C-terminal domain (PICP domain), a rod-like triple helical central domain and globular N-terminal domain (PINP domain)[4,5]. Folded collagen trimers are secreted out of the cell, where the PINP and PICP are cleaved off by ADMTS protease (PINP) and by bone morphogenic protein 1 (PICP)[6]. The released triple helical rod-like domains spontaneously polymerize into fibrils and are laterally cross linked

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by lysyl oxidase[7]. The fibrils provide strength to the extracellular matrix of the structural tissue. Major cell types capable of high level of type I collagen synthesis include fibroblasts, myofibroblasts, osteoblasts, smooth muscle cells and hepatic stellate cells (HSCs)[8–12].

REGULATION OF COLLAGEN SYNTHESIS

Constitutive and inducible collagen synthesis

Type I collagen is a relatively stable protein and turns over slowly with a half-life of 30–60 days. Since there is about 3–5 kg of type I collagen per average adult human, only about 40 g of type I collagen must be synthesized daily as the replacement synthesis. When estimated as a fractional synthesis rate (FSR, expressed as % synthesis per hour) collagen synthesis is slower than most proteins. The FSR of type I collagen was estimated to be 0.076 ± 0.063 % per h or about 2% per day and is in a good agreement with collagen half-life of 30–60 days[13]. This is substantially slower than, for example, the FSR of plasma proteins, which is about 0.5 % per h[14–16]. However, this dramatically changes where there is an acute demand for high collagen output, like in reparative fibrosis (wound healing, fractures), or in reactive fibrosis (pathological scarring of organs). When PICP and the N-terminal propeptide of type III procollagen (PIIINP) were measured in the wound fluid of surgical wounds the first increase in the PICP was observed at day 2 after wounding and it was increased 4-fold compared to day 1. PIIINP was first increased by 2.5 fold at day 3. On day 7 after wounding, the mean concentration of PICP was 380 times higher than that at day 1 and the mean concentration of PIIICP was 250 times higher. This indicates that after a profibrotic insult, activated fibroblasts and myofibroblasts can rapidly upregulate the synthesis of two major fibrilar collagens to achieve several hundred-fold higher synthesis than the constitutive synthesis rate.

Liver fibrosis is pathological state caused by diffuse, excessive accumulation of type I collagen in the liver[17]. In vitro studies using liver slices derived from fibrotic murine livers showed that fibrotic liver slices synthesized 16–30 fold more collagen than normal liver slices[18]. Hepatic stellate cells (HSCs) are liver cells responsible for excessive collagen synthesis in hepatic fibrosis[11]. Isolated HSCs upregulate their collagen expression by 50–100 fold within days after a profibrotic stimulus[19]. These examples illustrate that tissues that normally do not synthesize significant amounts of type I collagen have the ability to rapidly and dramatically increase the rate of collagen production. Elucidating how collagen expression can be rapidly altered is critical to understand the mechanism of reparative and reactive fibrosis. This review will focus on the RNA-protein interactions that are central to this mechanism.

Critical features of type I collagen synthesis

Type I collagen is a heterotrimeric protein that is in all tissues composed of two α1(I) polypeptides and one α2(I) polypeptide. The non-equivalence of three chains is important for lateral interactions between the adjacent helices and may determine the long-range axial order of the fibrils[20]. Collagen α1(I) chains have a propensity to form homotrimers, but a mechanism has evolved to prevent formation of homotrimers and assure exclusive synthesis of the heterotrimeric type I collagen. This is illustrated by rare mutations in humans, which result in a complete absence of α2(I) chain (21). Patients with complete absence of collagen α2(I) polypeptide synthesize the homotrimers of α1(I) chains and their phenotype ranges from hyper elasticity of skin and tendons, to cardiac valve prolapse, to severe osteogenesis imperfecta (brittle bones)[21,22]. Thus, synthesis of α1(I) homotrimers is compatible with life but is highly detrimental.

Similar phenotype was found in mice in which α2(I) gene had been inactivated (oim/oim mice)[23,24]. The oim/oim mice have reduced body size, smaller tendon bundles, osteopenia

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and decreased bone strength and secrete only homotrimers of type I collagen. The thermal stability of these homotrimers is lower than that of heterotrimers, indicating that their strength is insufficient to support the structural tissues[25]. These examples clearly indicate the importance of a mechanism that controls exclusive synthesis of collagen heterotrimers. This mechanism must coordinate translation of α1(I) and α2(I) polypeptides to increase their local concentration within the ER and to facilitate the chain registration in 2:1 ratio. If α1(I) and α2(I) polypeptides are translated randomly so that their registration depends on the rate of diffusion, a significant fraction of the homotrimers would form even in the presence of normal levels of α2(I) polypeptide.

Some patients with osteogenesis imperfecta have mutations in the C-terminal domain of α1(I) chains, affecting the amino acids responsible for chain registration. This results in slow folding of the triple helix and excessive posttranslational modifications of the polypeptides. The hypermodifictions are due to the prolonged access of the modifying enzymes to the unfolded chains[26,27]. This suggests that the rate of folding and the rate of modifications are in a dynamic equilibrium. The dependency of proper modifications on the rate of folding further supports the notion that collagen polypeptides must be synthesized in coordination.

Thus, in reparative or reactive fibrosis the cells not only produce 10–100 fold more of type I collagen, they also have to coordinate this process, such that only properly modified, heterotrimeric type I collagen is formed. The next sections will describe the RNA binding proteins involved in these processes.

RNA BINDING PROTEINS INVOLVED IN REGULATION OF TYPE I COLLAGEN EXPRESSION

Posttranscriptional regulation of type I collagen expression

Expression of any protein in the cell is regulated at multiple levels. The levels that determine the amount of a functional protein include transcription, mRNA splicing, export of the spliced mRNA into the cytoplasm, mRNA half-life in the cytoplasm, its translation efficiency and the stability of the produced protein. Whenever there is a large change in expression of a protein, it is likely that multiple steps are affected and that their cumulative effect is responsible for the dramatic change observed. Type I collagen is a classic example of such complex regulation.

Early efforts to understand the dramatic increase in type I collagen synthesis in wound healing and fibrosis concentrated solely on the transcriptional regulation[28–30]. The motivation behind these efforts had been a belief that there is a specific transcription factor that regulates expression of collagen genes. In the search for such a factor, functional elements in the promoter of collagen α1(I) gene were analyzed by generation of a series of transgenic mice harboring reporter genes driven by various segments of the promoter[30–32]. What emerged from these studies, however, was that it is not possible to reproduce quantitative and tissue specific aspects of type I collagen expression by utilizing only the transcription controlling elements. No collagen specific transcription factor could be identified and it became clear that additional levels of regulation must supersede the transcriptional regulation.

HSCs are an excellent model to study regulation of collagen expression. When isolated from normal rat livers and cultured in vitro, these cells undergo spontaneous activation from cells that produce low levels of type I collagen (quiescent HSCs) to cells that increase collagen expression 50–100 fold (activated HSCs) within 7 days[11,33,34]. To assess what type of regulation predominates in HSCs, the steady state level of collagen mRNA, the rate of

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transcription of collagen genes and the half life of collagen mRNAs were measured in quiescent HSCs and culture activated HSCs[19]. While the steady state level of collagen α1(I) mRNA was increased 50–100 fold, the rate of transcription of collagen α1(I) gene, as estimated by nuclear run-off assays, was increased only 3 fold. However, the half-life of collagen α1(I) mRNA was prolonged from 1.6h in quiescent HSCs to more than 24h in activated HSCs[19]. This indicated that stabilization of collagen mRNAs is the predominant mechanism, which contributes to the dramatically increased expression of the protein in HSCs. Subsequently, it was shown in other cell types that stabilization of collagen is critical to achieve high collagen expression after stimulation[34–40]. Because of the importance of posttranscriptional regulation of collagen expression, the search for RNA binding proteins that can specifically bind collagen α1(I) and α2(I) mRNAs became a critical endeavor.

Binding of αCP to the 3' UTR of collagen α1(I) mRNA

αCP was originally identified as an RNA binding protein that binds a C-rich sequence in the 3' UTR of α-globin mRNA[41]. α-globin mRNA has a half life of several days in reticulocytes, allowing continuation of hemoglobin synthesis long after the nucleus had been expelled from these cells. In α-globin mRNA, the C-rich sequence is located about 30 nt downstream of the stop codon. Mutation of this sequence resulted in rapid decay of the mRNA, suggesting that binding of αCP stabilizes α-globin mRNA[42,43]. In the 3' untranslated region (3' UTR) of collagen α1(I) mRNA there is a similar C-rich sequence, also located 30 nt downstream of the stop codon. The collagen C-rich sequence can bind αCP and can compete for its binding to the α-globin sequence[19]. Binding of αCP to collagen α1(I) mRNA is absent in quiescent HSCs and is present in activated HSCs[19]. In vitro mRNA decay experiments showed that depletion of αCP from the cell extracts accelerated the decay of collagen α1(I) mRNA, while knock down of αCP in activated HSCs decreased the steady state level of collagen α1(I) mRNA[44,45]. It was postulated that binding of αCP stabilizes collagen α1(I) mRNA in activated HSCs. However, no C-rich sequence was found in the 3'UTR of collagen α2(I) mRNA. Collagen α2(I) mRNA has a similar half life as α1(I) mRNA, but the mechanism responsible for its longevity has not been studied.

Later it was discovered that two additional mRNAs, tyrosine hydroxylase and lipoxygenase, also utilize the binding of αCP to the C-rich sequences in their 3' UTRs to prolong the half life[46]. Therefore, a common RNA stabilization mechanism; binding of αCP to the C-rich sequences located 3' to the stop codon, has been discovered. However, collagen α1(I) and α2(I) mRNAs have one additional and unique cis-acting sequence in their 5' UTR, the 5' stem-loop (5'SL), which proved to be essential for their posttranscriptional regulation.

Multiple polyadenylation signals in collagen mRNAs

Both collagen mRNAs have multiple polyadenylation signals that are utilized for 3' end formation. Collagen α1(I) mRNA has two polyadenylation signals separated by 1.1 kb, directing processing of a shorter transcript of 4.8 kb and a longer transcript of 5.9 kb. Both isoforms are expressed in HSCs in ratio 1:1. Collagen α2(I) mRNA also has two polyadenylation signals, but they are separated only by 10 nt and direct formation of a 5.4 kb transcript. 5' to the distal collagen α1(I) polyadenylation site and 5' to the each of collagen α2(I) polyadenylation signals there is a conserved sequence, termed the upstream sequence element (USE)[47]. The USE can influence polyadenylation efficiency and regulate formation of the shorter or longer versions of the transcripts. In collagen α2(I) mRNA the two closely positioned polyadenylation signals are in competition with each other. Mutation of the USE which precedes the polyadenylation signal decreased the efficiency of its utilization. The stimulatory effect of the USE was attenuated by a USE oligonucleotide, suggesting titration of trans-acting factors[47]. The identity of these trans-acting factors is

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unknown, but it can be envisioned that, by modulating such factors, the cell can alter the utilization of the poly-A signal and produce collagen transcripts of different lengths. This may have a profound influence on their stability and translatability.

5’ stem loop of collagen mRNAs

During the course of evolution sequence elements that are indispensable for function are conserved between distant species. Type I collagen appeared with the radiation of vertebrates, intriguingly in the 5’UTR of collagen α1(I) and α2(I) mRNAs of all vertebrate species there is a conserved sequence that is not found in invertebrate collagen genes. About 80 to 90 nucleotides from the cap and encompassing the start codon there is a stem-loop structure comprising of two stems flanking a central bulge[48]. This sequence is 48 nucleotides long, but differs only in 3 nucleotides between human and fish collagen mRNAs. It has been termed the collagen 5’ stem loop (5’SL)[49].

The extraordinary evolutionary conservation of the 5’SL suggests an important function. Although one early study was unable to find a function of this element[50], the later work employing HSCs provided compelling evidence that the 5’SL is critical for high collagen expression. The first hint about the importance of the 5’SL came from the experiments with molecular decoys[51]. A short RNA containing collagen 5’SL at the 5’ end and the binding site for the set of Sm proteins at the 3’ end was constructed. The 5’SL sequence was used to titrate the putative trans-acting factors involved in collagen expression, while packaging with the Sm proteins provided stability to the decoy particle[52]. When this decoy was expressed in HSCs collagen expression was decreased several fold, compared to the control decoy lacking the 5’SL[51]. This was the first demonstration of the functional significance of the 5’SL binding proteins in collagen expression by activated HSCs. Thus, one of the roles of the 5’SL is to tether the trans-acting factor(s) to collagen mRNAs.

The ultimate confirmation about the role of the 5’SL in collagen expression was obtained by creation of the 5’SL knock in mice. In these animals the 5’SL was mutated in the context of collagen α1(I) gene. The mutation only changed the nucleotides involved in the formation of the 5’SL, without affecting the coding region or the promoter of the α1(I) gene. The 5’SL in collagen α2(I) gene was intact, thus the homozygous mutant animals translated collagen α1(I) polypeptide from the mRNA without the 5’SL and collagen α2(I) polypeptide from the wt mRNA. These mice were viable, suggesting that collagen synthesis in embryonic development and housekeeping collagen synthesis in the adults had not been dramatically altered. However, when liver fibrosis was induced in the homozygous mutant animals, they developed only 20–30% of the degree of fibrosis seen in the control animals[53]. HSCs isolated from the mutant animals were unable to increase collagen expression upon activation in vitro, their collagen expression was several fold lower compared to HSCs isolated from control mice[53]. These findings confirmed the importance of the 5’SL in regulation of collagen expression in vivo.

The homozygous 5’SL mutant mice develop normally, indicating that sufficient amounts of type I collagen can be synthesized from α1(I) mRNA without the 5’SL. This reflects that the housekeeping collagen synthesis can be supported regardless of the presence of the 5’SL in one collagen mRNA. However, when a high demand for type I collagen arises, the 5’SL dependent collagen synthesis becomes critical. This has practical implications for development of antifibrotic drugs that would target the binding to the 5’SL. Such drugs would only affect the excessive collagen synthesis. Therefore, cloning of the 5’SL binding protein became of the utmost importance.

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LARP6 binds 5'SL of collagen mRNAs with high affinity and specificity

Expression cloning strategy was employed to clone the 5'SL binding protein. A plasmid cDNA library derived from human fibroblasts was amplified in pools and each pool was expressed in mammalian cells. The presence of binding activity to the 5'SL RNA was analyzed in the extracts of transfected cells by gel mobility shift assay. One pool containing the binding activity was identified and a single clone from this pool was isolated; it encoded for La ribonucleoprotein domain family member 6 (LARP6) protein[54]. LARP6 is a member of LARP protein superfamily, which has 6 members[55]. All LARP family members have the "La" domain, followed by an RNA recognition motif (RRM). The function of "La" domain is unknown, while RRMs of the La family members share a limited similarity with the RRMs of other RNA binding proteins. This classifies LARPs as RNA binding proteins. However, there are no reports that these proteins can bind any particular mRNA with high affinity. They may regulate translation of a subset of mRNAs, because knock down of LARP1 or LARP4b decreases the overall translation by 10–15%, but the mRNAs, translation of which depends of LARPs, have not been identified[56,57].

LARP6 is a unique family member, because it appears that it has diverged to specifically regulate expression of collagen mRNAs. LARP6 is found in plants and invertebrates, but the RRM of LARP6 of lower organisms differs from that of vertebrates[55]. Human LARP6 binds the 5'SL of collagen α1(I) and α2(I) mRNA with a Kd of 3–5 nM, as a sequence specific RNA binding protein[54]. LARP6 also binds a stem-loop present in collagen α1(III) mRNA (which encodes for type III collagen). This stem-loop is similar to that of collagen α1(I) and α2(I) mRNAs, but binding of LARP6 is of several fold lower affinity. No other RNA has been identified to which LARP6 can bind with high affinity, classifying it as the specific RNA binding protein of collagen mRNAs. LARP6 contacts five nucleotides within the single stranded bulge of the 5'SL (circled in Fig 1). Mutation of each individual nucleotide reduces the binding affinity by 3–4 fold, while the mutation of all five nucleotides abolishes the binding (our unpublished result). LARP6 needs both domains, the La domain and the RRM, to bind the 5'SL. This indicates that the high affinity and specificity of binding of LARP6 to the 5'SL is determined by multiple RNA protein contacts, involving two protein domains and five nucleotides of the 5'SL.

INTERACTIONS OF LARP6 THAT REGULATE TRANSLATION OF COLLAGEN mRNAs

Proteins that interact with LARP6

Knocking down of LARP6 by siRNA reduced collagen expression by 80%, indicating that it is an essential component of the collagen biosynthetic pathway[54]. Because LARP6 directly binds the 5'SL, it is likely that it functions as an adapter to tether other effectors to the collagen mRNAs. Immunoaffinity and RNA affinity pull down techniques were used to identify proteins that associate with LARP6. These proteins include nonmuscle myosin II, vimentin and RNA helicase A, the role of these proteins has been characterized in collagen expression and will be discussed here.

RNA helicase A is an essential cofactor for translation of collagen mRNAs

RNA Helicase A (RHA), also known as DEIH motif DHX9 helicase, is ubiquitously expressed and is a well conserved RNA binding protein with ATPase and RNA helicase activities[58,59]. Although there are some reports describing its role in gene transcription, the main role of RHA is in mRNA, splicing, export and translation. To become translationally competitive, some structured mRNAs recruit RHA to unwind the secondary structures in the 5' UTRs[60,61]. This is usually achieved by direct binding of RHA to some structural element in the 5'UTR of these mRNAs. In some retroviral mRNAs and JunD mRNA a structural

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element in the 5'UTR has been identified and termed the post-transcriptional control element (PCE). It consists of a stem-loop followed by GC-rich sequences[58,59,62]. RHA directly binds the PCE element, promotes its unwinding and increases the translation efficiency of these mRNAs.

Collagen α1(I) and α2(I) mRNAs can be regarded as poor translational substrates. Besides 5' SL, they also contain two short upstream open reading frames (uORFs), located between the cap and the 5'SL. In general, uORFs are inhibitory to translation, because they impede the scanning of small ribosomal subunit towards the start codon of the main frame[63–65]. RHA does not directly bind the 5'SL of collagen mRNAs, it is tethered to this structure by interaction with LARP6. When RHA was knocked down by siRNA, collagen α1(I) and α2(I) mRNAs were poorly loaded onto polysomes and were inefficiently translated[66]. The knock down of RHA did not change the overall polysomal profile, suggesting that general translation is not dependent on this protein, but only the translation of selected mRNAs; like collagen mRNAs or PCE containing mRNAs[66]. Thus, one of the roles of LARP6 in collagen expression is to tether RHA to collagen mRNAs to increase their translatability.

Interaction of LARP6 with nonmuscle myosin regulates translation of collagen mRNAs

Identification of nonmuscle myosin as one of the proteins that associates with LARP6 was a surprise, because there had been no reports on the role of motor proteins in collagen expression. Nonmuscle myosin II forms bipolar filaments, which interact with and move actin filaments[67–69]. It has two major isoforms, IIA and IIB that are expressed in cells exhibiting contractility or locomotion and has been extensively studied with respect to these functions. However, there have been no reports on the role of nonmuscle myosin in mRNA translation. Collagen mRNAs immunoprecipitate with nonmuscle myosin and their association depends on the expression of LARP6. Disruption of the integrity of nonmuscle myosin filaments had dramatic effects on type I collagen synthesis[70]. ML7 is a drug that inhibits myosin light chain kinase (MLCK) and inhibition of MLCK results in depolarization of nonmuscle myosin filaments[71]. Depolymerization of nonmuscle myosin filaments can also be achieved by overexpression of a MLCK mutant that lacks the catalytic activity (kinase-dead MLCK)[72]. When nonmuscle myosin filaments were disrupted in lung or skin fibroblasts by either of these methods, the cells failed to secrete heterotrimeric type I collagen. Instead, they secreted only the homotrimers of α1(I) chains, while the secretion of α2(I) chain was barely detectable. This was associated with increased intracellular degradation of both chains. The level of collagen mRNAs was not changed, suggesting that collagen mRNAs were translated, but the collagen polypeptides made were unable to fold properly. One interpretation from these experiments was that in the absence of nonmuscle myosin filaments collagen mRNAs are translated randomly and that the rate of diffusion in the ER limited the registration of collagen chains. The result is formation of homotrimers and intracellular degradation of the chains that could not find the folding partners.

In a mouse model of cardiac fibrosis, the fibrotic foci were found only in the areas of the heart which re-expressed nonmuscle myosin[73]. Likewise, in liver fibrosis HSCs that activate into collagen producing cells dramatically increase expression of nonmuscle myosin. This supports the notion that, by binding to collagen mRNAs, nonmuscle myosin filaments coordinate translation of the collagen polypeptides. This increases formation of collagen fibrils several hundred fold.

Vimentin filaments stabilize collagen mRNAs

Tobramycin RNA affinity chromatography was a method developed to purify loosely associated splicing complexes[74,75]. When this method was used to purify complexes associated with 5' SL, vimentin was one of the proteins purified[76]. Vimentin is a marker of

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cells of mesenchymal origin and forms intermediate filaments, but its role in cells has been elusive[77–79]. Vimentin knock out mice have normal phenotype, except they exhibit delayed wound healing[80]. Collagen mRNAs immunoprecipitate with vimentin filaments and RNA-FISH experiments showed that collagen α1(I) and α2(I) mRNAs are associated with vimentin filaments in vivo. Knock down of LARP6 by siRNA or mutation of the 5'SL abrogated the interaction of collagen mRNAs with vimentin filaments, suggesting that LARP6 mediates the interaction. The La domain of LARP6 is necessary and sufficient for interaction of LARP6 and vimentin and this was the first report of a function of the La domain[76]. Vimentin knock out fibroblasts produce reduced amounts of type I collagen compared to wt cells. The half life of collagen mRNAs in vimentin knock out fibroblasts is about 6h, compared to 12h in wt fibroblasts[76]. This suggested that the decreased collagen expression is due to decreased stability of collagen α1(I) and α2(I) mRNAs. Treatment of cells with a vimentin depolymerizing agent or overexpression of a dominant negative form of desmin, which disrupts vimentin filaments[81], reduced type I collagen expression. When the cells were treated with puromycin to dissociate polysomes, the amount of collagen mRNAs that can be immunoprecipitated with vimentin increased, suggesting that vimentin sequesters collagen mRNAs that are not actively engaged in translation[76].

The finding that vimentin filaments stabilize collagen mRNAs was a rationale to use Withaferin A (WF-A), another drug that can disrupt vimentin filaments, to attenuate cardiac fibrosis[82]. WF-A disrupted vimentin filaments at concentrations of 0.5–1.5 μM in cardiac fibroblasts and reduced 3 fold the half-lives of collagen α1(I) and α2(I) mRNAs. In addition, WF-A inhibited TGF-β1 induced phosphorylation of TGF-β1 receptor I, Smad3 phosphorylation and transcription of collagen genes[82]. When administered into mice at 4 mg/kg daily for 2 weeks, WF-A reduced isoproterenol-induced myocardial fibrosis by 50%[82]. These findings provided a proof of principle that targeting vimentin filaments can be an effective antifibrotic therapy, including but not limited to cardiac interstitial fibrosis.

CONCLUSIONS

Type I collagen is the most abundant protein in human body, but in an adult organism its fractional synthesis rate is low. However, in reparative or reactive fibrosis the cells are able to rapidly upregulate collagen synthesis several hundred fold. Binding of LARP6 to the conserved 5' SL structure in collagen mRNA is critical for this regulation. LARP6 associates collagen mRNAs with filaments composed of nonmuscle myosin and tethers RHA to promote their translation. It also associates nontranslated collagen mRNAs with vimentin filaments to prolong their half life. Collagen producing cells develop robust nonmuscle myosin filaments when activated by wounding or by fibrosis. Nonmuscle myosin also provides the driving force for cell migration towards the site of the injury. Thus, by acquiring the motor filaments for migration, the cells also acquire a platform to support translation of collagen mRNAs. This couples two fundamental processes of wound healing; cell migration and scar deposition. Further elucidation of these key steps in collagen biosynthesis will contribute to development of specific antifibrotic drugs targeting LARP6 dependent mechanism of collagen synthesis.

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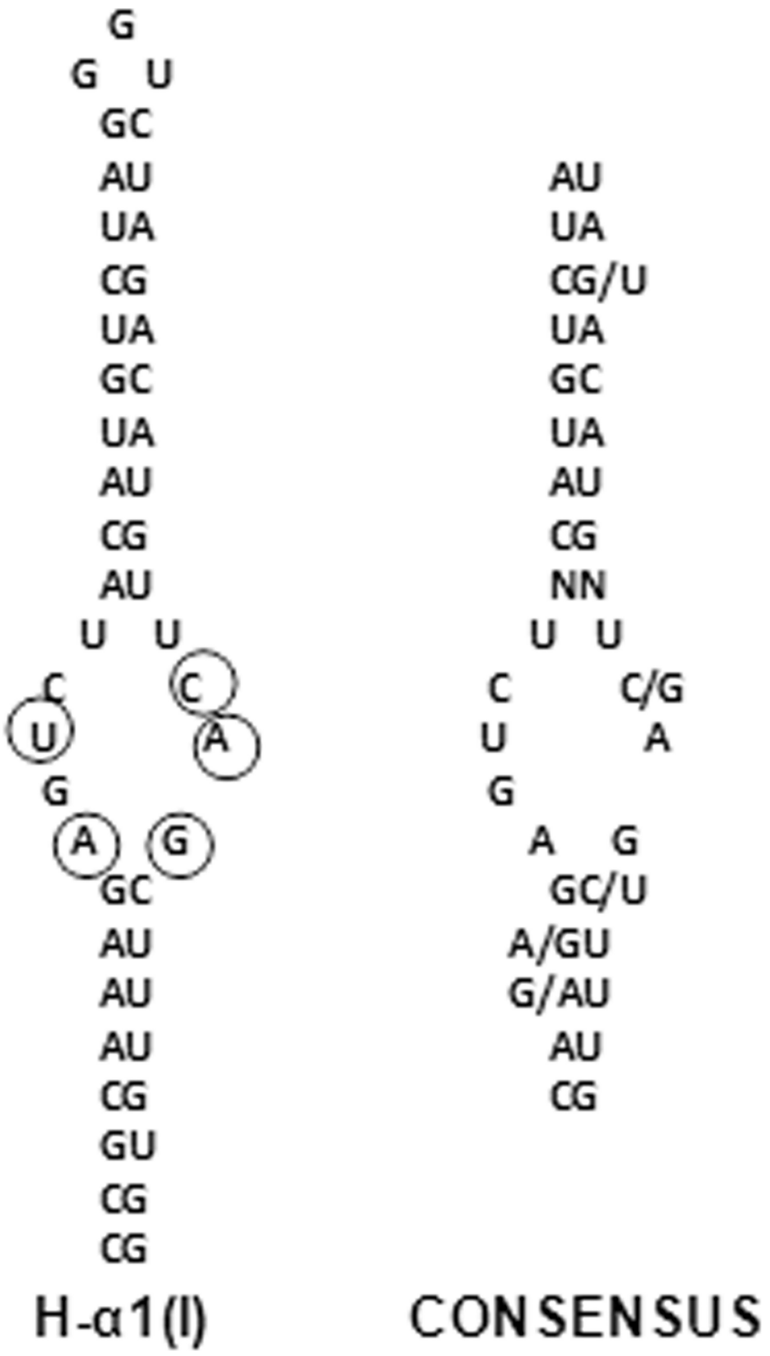

Figure 1.
5’SL of human collagen α1(I) mRNA (H-α1(I), left panel). Nucleotides involved in binding LARP6 are circled. Right panel: the consensus sequence of 5’SL derived from all vertebrate collagen α1(I) and α2(I) mRNAs.

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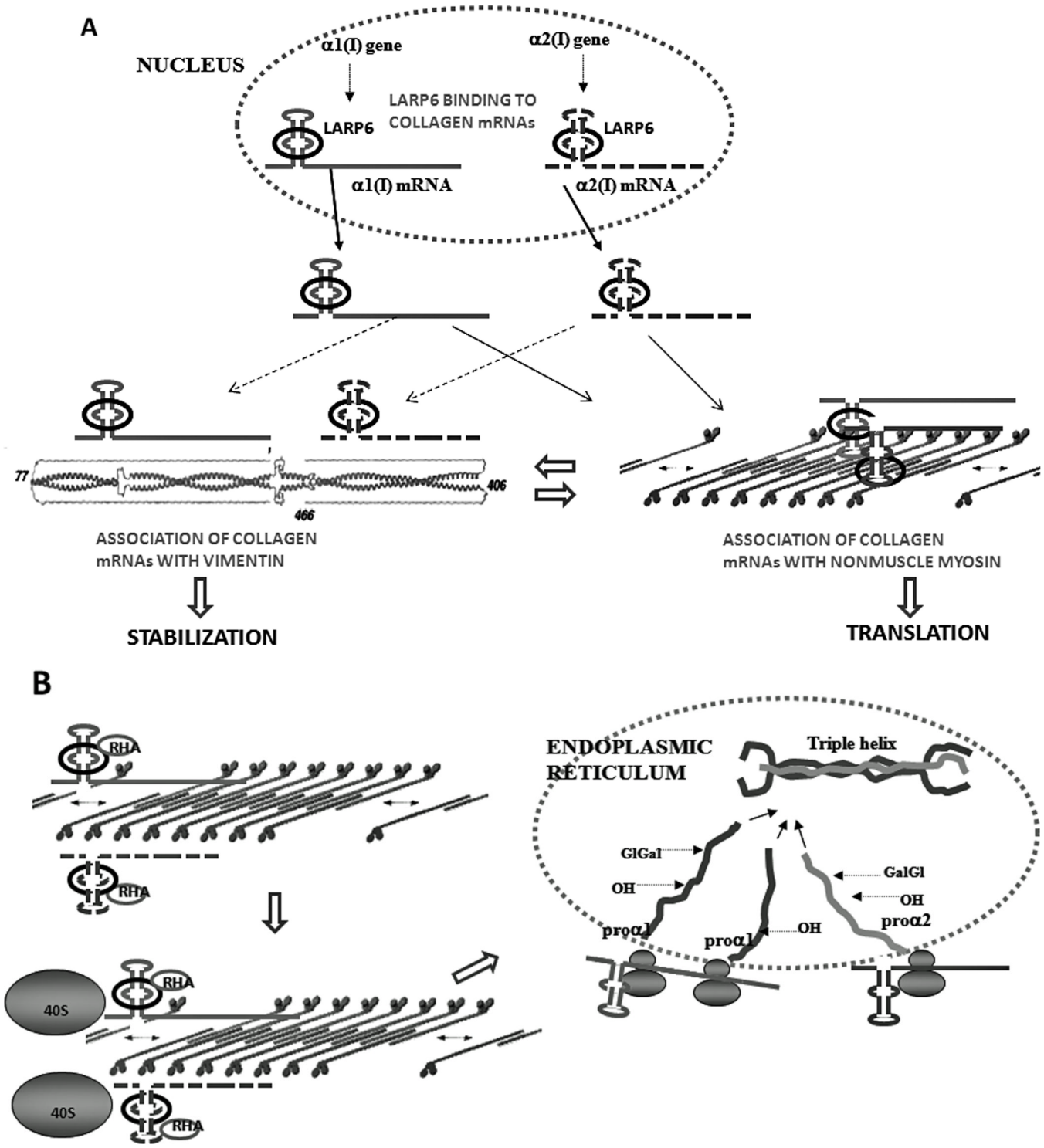

Figure 2.

LARP6 dependent synthesis of type I collagen. A. Association of collagen mRNAs with cytoskeletal filaments. The process starts with binding of LARP6 to collagen mRNAs in the nucleus, what may facilitate their export to the cytoplasm. In the cytoplasm LARP6 associates collagen mRNAs with vimentin filaments or with nonmuscle myosin filaments.

Shuttling of collagen mRNAs between these filaments is possible. Binding to vimentin stabilizes collagen mRNAs, while nonmuscle myosin filaments support their translation. B. Coordinated translation of collagen mRNAs. The integrity of nonmuscle myosin filaments is necessary for translation to initiate on collagen α1(I) and α2(I) mRNAs in coordination. LARP6 recruits RHA to unwind the 5'SL and collagen mRNAs are targeted to the membrane of the endoplasmic reticulum by the signal recognition particle or some other mechanism. Subsequent translation elongation takes place, resulting in increased local concentration of collagen polypeptides for productive folding into the collagen heterotrimer.

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