Minireview

Membrane traffic fuses with cartilage development

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Received 30 June 2003; revised 21 July 2003; accepted 21 July 2003

First published online 5 August 2003

Edited by Felix Wieland

Abstract The ability of cells to synthesize and secrete proteins is essential for numerous cellular functions. Therefore, when mutations in one component of the secretory pathway result in a tissue-specific defect, a unique opportunity arises to examine the molecular mechanisms at play. The recent finding that a defect in the protein sedlin, whose yeast counterpart is involved in the first step of the secretory pathway, leads to a cartilage-specific disorder in humans raises numerous questions and interesting possibilities for understanding both the pathobiology involved and the role of membrane traffic in normal cartilage development.

Key words: Membrane traffic; Spondyloepiphyseal dysplasia tarda; Transport protein particle; Sedlin

1. Introduction

Some fields of research spawn natural alliances. Such is the case, for example, with the fields of apoptosis and cancer research, inexorably linked through the study of both cell cycle regulation and the protein p53. In other areas, such cross-disciplinary studies between seemingly related fields never seem to rapidly materialize. This appears to be the case with the fields of membrane traffic and cartilage development. Indeed, it is well established that cartilage proteins, including the abundant type II collagen, are synthesized intracellularly and utilize the secretory pathway for traveling to their ultimate destination. Fracture callus, an intermediate in bone healing, requires, among other events, the deposition of collagen in the form of cartilage. Given the large size of the collagen macromolecules that are secreted, these processes present a unique trafficking problem for the cell. Chondrocytes, therefore, might serve as an interesting model system in which to study the movement of such large molecules through the pathway and to examine whether alternative mechanisms, and perhaps other proteins, are employed. The paucity of literature connecting these two areas is, therefore, surprising.

That, however, may be changing. Recent work has demonstrated a strong and undeniable connection between membrane traffic and cartilage development which should allow for some interesting cell biological questions to now be addressed. This article will summarize that link, focusing mainly on the first stage of the secretory pathway, and will present some of the unresolved issues and lay out some of the challenges for further investigation.

2. Cartilage and ER–Golgi transport

Articular cartilage is composed of both collagenous and non-collagenous proteins [1]. Of the collagen family, type II collagen is the predominant form in cartilage [1,2]. Synthesis of these proteins is initiated at the rough endoplasmic reticulum (ER) where, upon translocation into the ER, the proteins receive their initial carbohydrate moieties. In the case of collagen, the protein receives proline modifications and is assembled into a triple helix bundle before leaving the ER as a large macromolecule. Transport to the Golgi complex allows for further modification of the sugar residue side chains ultimately leading to the mature protein in a form ready for secretion into the extracellular space. As some of the transport vesicles carrying these components are relatively small (~50 nm in diameter), a mechanism to transport any components greater than this size must be employed. This is particularly an issue for many of the collagens (including type II collagen) and some of the proteoglycan core proteins, whose lengths range into the hundreds of nanometers [3]. The movement of procollagen type I through the Golgi complex has been studied kinetically and at the ultrastructural level [4] and it was postulated that the protein travels through the Golgi by a progressive maturation of the cisternal stacks ('cisternal maturation' model). In another study, using an artificially induced protein aggregate to follow traffic through the Golgi, Volchuk et al. [5] described much larger vesicles than had been previously identified. Such so-called megavesicles appeared at the edges of the Golgi cisternae and little of the marker aggregate was detected in more internal regions of the stack. Similar distensions of the Golgi were also visualized for the large lipoprotein apolipoprotein E [6]. Given the small size of ER-derived transport vesicles (also ~50 nm in diameter), it is not entirely clear how these large molecules transit from the ER to the Golgi. One possibility is that vesicular-tubular clusters, which are on average 400 nm in diameter [7], may act as carriers. Recently, it has been shown that procollagen type I is sorted away from several other proteins early after exit from the ER suggesting that large molecules may be directed to the Golgi by a different mechanism than smaller proteins [8].

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Abbreviations: ER, endoplasmic reticulum; MBP-1, c-*myc* promoter binding protein-1; SEDL, spondyloepiphyseal dysplasia tarda; TRAPP, transport protein particle

0014-5793/03/$22.00 © 2003 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)00854-8

Table 1
Components of yeast and mammalian TRAPP

Saccharomyces cerevisiae TRAPP I subunit	Mammalian orthologue identified in EST database [12,13]	Orthologue known as	Mammalian orthologue precipitates with tagged bet3 [15]
Bet5p	Yes	MUM-2 [39]	Yes
Trs20p	Yes	Sedlin [21]	Yes
Bet3p	Yes		Yes
Trs23p	Yes	Synbindin [35]	Yes
Trs31p	Yes		No
Trs33p	Yes		Yes
Trs85p	No		Yes

Traffic between the ER and Golgi has been well studied in both yeast and mammalian systems and many of the components involved have been identified (see [9] and references therein). In general, the ER-derived vesicles must tether and dock with the Golgi before fusing with it. Studies in yeast have identified a multiprotein complex called transport protein particle I (TRAPP I) as one of the most upstream factors required for tethering these vesicles to the Golgi [10]. TRAPP I is composed of seven polypeptides with no significant homology to any protein of known function (Table 1). Besides tethering the vesicle to the *cis* side of the Golgi complex, TRAPP I also facilitates the exchange of nucleotide on a protein called Ypt1p [11]. By converting Ypt1p to its 'activated form', TRAPP I indirectly allows for the recruitment of additional factors (effectors of Ypt1p) to the site of the tethered vesicle, setting in motion other poorly defined events which ultimately allow the vesicle to fuse with the Golgi.

While little functional characterization has been done on the mammalian TRAPP complex, mammalian cells do possess orthologues to many of the yeast subunits (Table 1) [12,13] which are assembled into a high molecular weight complex [14,15].

3. The 'missing' link

Spondyloepiphyseal dysplasia tarda (SEDL) is a skeletal disorder leading to dysplasia of the large joints. An X-linked form of this disorder has been described which manifests clinically in affected males as short stature, barrel-chested appearance, dysplasia of the large joints and narrowed intervertebral disc space [16,17]. The disorder, which presents at ~10 years of age as mild joint pain, often necessitates hip replacement before the third decade of life [18]. Initial mapping linked the disease gene to the X chromosome at the Xp22 region [19,20]. Subsequent work showed that the gene, whose protein is called sedlin, was truncated in three affected males from unrelated lineages [21]. Surprisingly, sedlin is the human orthologue of the TRAPP I subunit Trs20p (see [12]). A more recent study examined 27 patients clinically diagnosed with SEDL and a variety of mutations in sedlin were found in all affected males including frameshifts (resulting in truncations), mutations predicted to disrupt normal mRNA splicing, as well as nonsense and missense mutations [18].

Recently, the crystal structure of sedlin was solved [22]. The structure shows an unusually large number (nearly 15%) of apolar residues exposed at the surface of the protein, implying that this subunit is involved in multiple protein–protein interactions. Indeed, several hydrophobic pockets and grooves have been described on the protein as well as a stretch (^{114}MNPFY^{118}) of surface-exposed apolar residues. Curiously, this stretch includes the sequence NPF which has been shown to be involved in protein–protein interactions with targets containing the epsin homology (EH) domain [23]. It would be of interest, therefore, to determine if sedlin in fact interacts with an EH-domain-containing protein and whether such a protein plays a role in cartilage development. The crystal structure also suggests that a missense mutation (D47Y) identified in a patient with SEDL, which removes a charge from a surface-exposed residue, may interfere with a protein–protein interaction. Thus, the identification of proteins which interact with this region of the molecule would be of great interest. Finally, a structural similarity between sedlin and the regulatory domains of the SNAREs ykt6 and sec22b was noted. The SNAREs are a family of proteins found on donor and acceptor membranes which are required for membrane fusion. This raises the possibility of SNARE–sedlin interactions as well.

Yeast two-hybrid assays have shown that Trs20p and sedlin interact with chloride intracellular channels [24], c-*myc* promoter binding protein (MBP)-1 [25] and the yeast proteins Bet3p [26] and Trs31p [27]. The interaction between Trs20p and the latter two proteins is not surprising as all these pro-

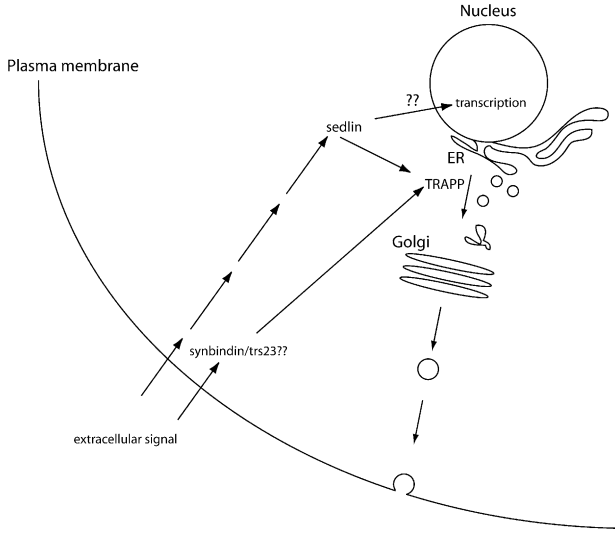

Fig. 1. The function of the multiprotein complex TRAPP I containing Trs20p, the yeast orthologue of sedlin, in ER–Golgi traffic is well established [10]. Sedlin is found in a high molecular weight complex in HeLa cells as well as in an unassembled state [14], perhaps allowing it to be involved in multiple processes. Genetic interactions [25] and subcellular localization [28] indicate sedlin may enter the nucleus and might be involved in the regulation of gene transcription as well. The trs23 subunit, also known as synbindin, interacts and co-localizes with a cell surface protein involved in transducing extracellular signals [35] and may be involved in linking intracellular traffic to extracellular events.

teins are components of TRAPP I. The interaction of sedlin with the transcriptional repressor MBP-1 is interesting in light of the finding suggesting that truncated sedlin accumulates in or around the nucleus [28]. This latter result suggests that full-length sedlin may in fact cycle into and out of the nucleus during its itinerary which would make interaction with nuclear proteins a real possibility. Sedlin does not appear to have the leucine-rich consensus sequence for nuclear export, found in ~35% of proteins which exit the nucleus [29]. However, in this respect it is interesting to note that three of the final eight amino acids at the carboxy-terminus of sedlin are leucines which would be absent in all of the documented SEDL truncations, thus possibly explaining accumulation of the truncated form in the nucleus. Alternatively, truncated sedlin may fail to interact with another protein that would help shuttle it out of the nucleus. Nevertheless, the interaction with MBP-1 raises the intriguing possibility of a dual function for sedlin in both membrane traffic and the regulation of gene expression (Fig. 1), perhaps helping to explain the pathological basis for SEDL. It would be of interest to determine whether sedlin interacts with other, perhaps chondrocyte-specific, transcriptional repressors (see below).

4. Future perspectives

Although a trafficking defect may be the more favored explanation for the etiology of SEDL (see below), several interesting cell biological questions regarding membrane traffic and cartilage development arise. First, if sedlin is ubiquitously expressed [21,30], why does mutation of the protein lead to a tissue-specific disorder? To address this question, it will be of interest to assess ER–Golgi traffic in SEDL patients. In particular, the trafficking of the proteins destined for the cartilage should be examined. One possibility is that sedlin is intimately involved in recognizing a particular class of ER-derived transport vesicles enriched in these proteins. Recall that the 'standard' ER-derived vesicle is ~50 nm in diameter while some of the cartilage proteins are too large to fit into such vesicles. Whether there is a second class of ER-derived vesicles in chondrocytes is mere speculation. It should be noted, however, that since two classes of vesicles have been reported to bud from the ER in the lower eukaryotic organism *Saccharomyces cerevisiae* [31] it is not unreasonable to speculate that higher eukaryotes may have evolved alternative transport mechanisms from the ER which may vary from cell to cell. Interestingly, an early sorting event after ER exit has been noted in mammalian cells resulting in the segregation of procollagen type I from the vesicular stomatitis virus G protein and ERGIC53 [8]. It is tempting to speculate that sedlin might be directly involved in recognition of these procollagen-containing transport intermediates (see [32]). Indeed, the extracellular matrix from one SEDL patient was reported to contain collagen fibrils that were shorter than normal and frayed as well as fibrils of varying diameter [32], suggestive of a defect in the normal secretion of this macromolecule in this patient. A comparison between the secretion of collagen and that of other smaller proteins from this patient would be of interest.

Another possibility for the tissue-specific defect in SEDL patients could lie in the TRAPP subunit composition. The arrangement of the subunits within the complex could vary between tissues, with sedlin being more heavily represented in the complex in chondrocytes. A similar situation is found in yeast where, in this case, altering the TRAPP I subunit composition changes the activity of the complex (now referred to as TRAPP II) [10]. Generation of highly specific antibodies to each of the subunits will help to address this possibility.

Second, why is SEDL a late onset disorder and not manifested during initial formation of the embryonic cartilaginous network, a precursor to certain skeletal elements? A possible explanation for this question lies in the numerous pseudogenes for sedlin in the genome. One in particular (a retropseudogene on chromosome 19) is transcribed [21,25,28]. The pseudogene is predicted to encode a full-length protein identical to that found on the X chromosome. However, it has yet to be demonstrated whether the mRNA is translated in vivo. If so, one might speculate that this retropseudogene provides redundancy early in development and that transcription and/or translation of this retropseudogene is turned off later in development, leaving the sole expressed copy of sedlin originating from the X chromosome. If truncated sedlin fails to interact with MBP-1 then, in SEDL patients, this might lead to the clinical manifestations described above. In this respect it is noteworthy that carrier females appear to be asymptomatic (see [18]) indicating that only one copy of the wild-type protein is sufficient for sedlin function. This result would also provide the precedent for future studies aimed at examining the developmental regulation of membrane trafficking factors.

Third, is the interaction between sedlin and MBP-1 of physiological significance? It is not unreasonable to speculate that chondrocytes contain tissue-specific transcriptional regulators, given that both osteoblasts and chondroblasts possess transcription factors such as Osf2/Cbfa1 [33] and Sox9 [34], each playing a key role in tissue-specific growth. If sedlin interacts with chondrocyte-specific factors involved in transcription, then the exciting possibility exists that sedlin might be involved in both membrane traffic and gene expression (Fig. 1). This would not be the first TRAPP subunit purported to function outside of the complex. Another TRAPP subunit (trs23), designated synbindin [35], was shown to bind to cell surface heparin sulfate proteoglycans of the syndecan family and is reportedly involved in neuronal dendritic spine formation. Interestingly, syndecan-2 plays a role in osteoblast signal transduction [36] and is expressed during bone development and osteoblast differentiation [37] while syndecan-3 is believed to transduce signals in chondrocyte development [38]. In light of these findings, a more general question could be asked: do TRAPP subunits function outside the complex to somehow link other cellular events to membrane traffic (Fig. 1)? This is indeed an intriguing possibility which requires further examination. Perhaps, then, it is noteworthy that preliminary evidence indicates sedlin is a phosphoprotein (unpublished observation). Determination of the physiological function of the phosphorylation site(s) may help begin to unravel the answer to this question.

5. Conclusion

Although a defect in membrane traffic may be the most straightforward explanation for the disease state, tying together recent literature indicates other factors may come into play. The growing literature suggests that sedlin function (and perhaps that of other TRAPP subunits) may not be restricted to membrane traffic. The intersection where sedlin now stands indicates other areas of research such as signal transduction

and, perhaps, gene expression may yet become involved. To examine the multifunctional role of sedlin, it will be necessary to combine a variety of tools available to the developmental cell biologist, such as electron microscopy, knockout and transgenic mouse models, and RNA interference, with studies of subunit composition/assembly, protein–protein interactions and standard assays of membrane traffic. In this way, we will gain a better understanding of the link between cartilage development and protein secretion in disease and health.

Acknowledgements: I am grateful to Drs. Susie Tenenhouse, Dong-Wook Kim, Kathy Baynton, Janet Henderson and David Goltzman for helpful discussions and comments on the manuscript.

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