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Review

Trafficking mechanisms of extracellular matrix macromolecules: Insights from vertebrate development and human diseases

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ABSTRACT

Cellular life depends on protein transport and membrane traffic. In multicellular organisms, membrane traffic is required for extracellular matrix deposition, cell adhesion, growth factor release, and receptor signaling, which are collectively required to integrate the development and physiology of tissues and organs. Understanding the regulatory mechanisms that govern cargo and membrane flow presents a prime challenge in cell biology. Extracellular matrix (ECM) secretion remains poorly understood, although given its essential roles in the regulation of cell migration, differentiation, and survival, ECM secretion mechanisms are likely to be tightly controlled.

Recent studies in vertebrate model systems, from fishes to mammals and in human patients, have revealed complex and diverse loss-of-function phenotypes associated with mutations in components of the secretory machinery. A broad spectrum of diseases from skeletal and cardiovascular to neurological deficits have been linked to ECM trafficking. These discoveries have directly challenged the prevailing view of secretion as an essential but monolithic process. Here, we will discuss the latest findings on mechanisms of ECM trafficking in vertebrates.

Contents

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1. Introduction

Extracellular matrix (ECM) is a complex non-cellular structure synthesized by all tissues and is composed of water, proteins, and polysaccharides, as well as mineral deposits in skeletal tissues (Bosman and Stamenkovic, 2003). ECM composition is unique to each tissue and is deposited by fibroblasts or other specialized cells. For example, epithelial cells secrete basement membrane proteins, such as collagens, fibronectin and laminin, whereas chondrocytes and osteocytes secrete type II and type I collagens that are characteristic of mature cartilage and bone, respectively (Gay et al., 1976; Li et al., 1995; Reddi et al., 1977). Large structural ECM proteins are typically fibrillar, including collagens, fibronectin, and laminins. Collagens, for example, constitute over 30% of a total protein mass in multicellular organisms (Ishikawa and Bachinger, 2013) and are rapidly secreted during development or in response to pathological conditions such as wound healing after tissue injury (e.g. skin damage, myocardial infarction, liver cirrhosis) (Cleutjens et al., 1995; Clore et al., 1979; Gay et al., 1975; Pinzani et al., 2011).

The rapid secretion of large cargos such as collagens requires unique regulatory mechanisms to assure availability of specialized transport machinery (Melville et al., 2011; Saito et al., 2009a). Procollagen has been extensively used as a model-cargo in secretory pathway studies (Arnold and Fertala, 2013; Bonfanti et al., 1998; Ishikawa and Bachinger, 2013; Stephens and Pepperkok, 2002). As with all ECM proteins, procollagen is synthesized and initially post-translationally modified in the endoplasmic reticulum (ER), from which it is transported in a COPII (coat protein II complex)-dependent manner to the ER-to-Golgi intermediate compartment (ERGIC) *en route* to the Golgi complex, where further post-translational processing occurs (Canty and Kadler, 2005). Procollagen is then transported in tubular carriers to be secreted to the extracellular space, where it is cleaved and assembled into higher-order structures (Arnold and Fertala, 2013; Ishikawa and Bachinger, 2013; Polishchuk et al., 2003, 2009).

The first leg of this journey is export from the ER, which is mediated by the COPII complex (Fig. 1). Pioneering work using yeast genetics first identified 23 genes whose products are required for secretory activity (Kaiser and Schekman, 1990; Novick et al., 1980). Among them were components of the COPII complex (Barlowe et al., 1994). COPII formation is initiated when the cytoplasmic GTPase Sar1 undergoes a conformational change upon GTP binding and associates with the ER membrane (Barlowe et al., 1993; Kuge et al., 1994; Nakano and Muramatsu, 1989). Sar1 then recruits Sec23/Sec24 heterodimers to form the "inner coat" complex (Bi et al., 2002; Matsuoka et al., 1998). Two additional ER associated proteins, Sec12 that acts as a GEF (guanine nucleotide exchange factor) for Sar1 (Barlowe and Schekman, 1993) and Sec16 that is a large scaffold protein shown to associate with ER Exit Sites (Connerly et al., 2005; Espenshade et al., 1995; Watson et al., 2006) contribute to initiation of vesicle formation. While Sec23 serves as GAP (GTPase activating protein) for Sar1, resulting in coat dissociation from the vesicle membrane (Yoshihisa et al., 1993), Sec24 acts as a cargo adaptor by selecting distinct proteins for ER exit (Miller et al., 2002). Assembly of the inner coat is followed by recruitment of Sec13–Sec31 heterotetramer of the "outer coat" complex, which is thought to stabilize the coat (Bhattacharya et al., 2012; Bi et al., 2007; Copic et al., 2012; Stagg et al., 2006; Tang et al., 2000). The molecular nature of these processes has been reviewed elsewhere (Brandizzi and Barlowe, 2013; Szul and Sztul, 2011).

Unlike the baker's yeast genome (*Saccharomyces cerevisiae*) that harbors single copies of these essential genes, vertebrate genomes have an expanded repertoire of COPII genes, including Sar1a and Sar1b (Jones et al., 2003; Loftus et al., 2012), Sec23a and Sec23b (Paccaud et al., 1996; Wadhwa et al., 1993), Sec24A, Sec24B, Sec24C and Sec24D (Tang et al., 1999), Sec13 (Swaroop et al., 1994), Sec31a and Sec31b (Stankewich et al., 2006; Tang et al., 2000), (Fig. 1). Gene multiplication of the coat components might have been evolutionarily driven by expansion of the genomes to accommodate novel extracellular matrix proteins and more complex body plans.

Many of the additional COPII paralogs seem to be specific to vertebrates and might be associated with unique functions that are essential for vertebrate development, including organ structures that are supported by diverse types of basement membranes and an internal skeleton primarily composed of mineralized ECM of cartilage and bone (Braasch and Postlethwait, 2012; Forster et al., 2010; Norum et al., 2010). Thus it is not surprising that many loss-of-function mutations in the trafficking machinery components result in skeletal dysmorphology.

Here we will discuss cargo- and tissue-specific functions of the COPII machinery, post-translational modifications, phosphorylation and ubiquitylation of COPII proteins, and the effects they have on vesicle biogenesis. In addition we will discuss auxiliary proteins such as cargo receptors and guide proteins that were shown to assist in loading of ECM macromolecules into vesicular carriers. Finally, one of the most intriguing unanswered questions in regulation of secretion is transcriptional control of the secretory machinery. Most coat genes are ubiquitously expressed but are enriched in specific tissues at defined developmental time points or in pathological conditions. However, little is known about transcriptional control of secretion with only a single factor of the OASIS family, Creb3L2, implicated in the process so far.

In this review we will focus on recently discovered trafficking mechanisms in the initial leg of the secretory pathway, from the ER to Golgi, by highlighting studies of vertebrate model organisms and human genetic mutations.

2. ER-to-Golgi transport is facilitated by coat protein II (COPII) vesicular carriers

2.1. Cargo selection by Sec24 components of the inner coat

Tandem genome duplication expanded the ancestral Sec24 gene to two syntenic groups, one of Sec24A and Sec24B and the second of Sec24C and Sec24D (Tang et al., 1999). The four genes are highly divergent in sequence between the two groups (20% similarity) and approximately 50% similar between each pair, but each paralog is highly conserved within vertebrates (up to 90% sequence similarity between fish and human) (Sarmah et al., 2010). Pioneering work on transport signal recognition by the Sec24 paralogs using model cargo in Sec24-depleted HeLa cells revealed for the first time selectivity and redundancy in the cargo selection process (Wendeler et al., 2007).

Recent *in vivo* evidence obtained from phenotype-driven genetic screens in zebrafish, medaka and mouse have begun to uncover the complexity of Sec24-based cargo selection. To date, only Sec24D has been directly implicated in ECM secretion. The zebrafish mutant *bulldog*/*sec24d* fails to secrete type II collagen and matrilin from chondrocytes, fibroblasts and notochord sheath cells, leading to severe craniofacial dysmorphology, short body length and kinked pectoral fins (Sarmah et al., 2010). This phenotype is largely recapitulated by the *vbi*/*sec24d* medaka mutant carrying a nonsense mutation predicted to truncate a C-terminal portion of Sec24D protein (Ohisa et al., 2010). In both zebrafish and medaka, Sec24D-deficient chondrocytes accumulate type II collagen in distended rough endoplasmic reticulum (rER). However, other ECM and transmembrane proteins appear to be trafficked normally to the extracellular space and plasma membrane, including fibronectin, cadherin, and β1-integrin (Table 1). In mice, however, gene-trap mediated knockout of *Sec24D* gene leads to pre-implantation lethality with no discernible phenotype in a