Tissue and Cell 55 (2018) 31–38

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Pre-embedding labeling for subcellular detection of molecules with electron microscopy

Elena V. Polishchuk[a,b], Roman S. Polishchuk[a,*]

[a] Telethon Institute of Genetics and Medicine (TIGEM), Via Campi Flegrei 34, Pozzuoli, Naples, 80078, Italy
[b] ITMO University, Kronverkskiy pr., 49, St. Petersburg, 197101, Russia

ARTICLE INFO

Keywords:
Electron microscopy
Pre-embedding
Gold labeling
Peroxidase labeling

ABSTRACT

Labeling of specific molecules for electron microscopy provides an extremely powerful tool to investigate subcellular distribution of proteins, lipids and sugars with resolution of a few nanometers. Moreover, this technology offers a unique opportunity to see how the pattern of these molecules changes in different physiological or pathological conditions. Pre-embedding labeling evolved as one of the easiest method for molecule detection in electron microscopy. It was named "pre-embedding" because the procedure involves a labeling step before the embedding of the specimen in the resin, its subsequent sectioning and analysis of sections under the electron microscope. Here we review different strategies and technical tips of the pre-embedding procedure, the potential of this method for detection and quantification of molecular components at the ultrastructural level, and the integration of the pre-embedding approach with rapidly developing light and electron microscopy technologies.

1. Introduction

Scientific researchers in the 21 st century should consider themselves extremely fortunate. Significant breakthroughs in a wide range of important biological questions have been achieved through the development of a vast number of revolutionary techniques such as GFP video-imaging, genome editing and next generation sequencing. Importantly, the combination of traditional and innovative methods enables researchers to choose the optimal strategy to address particular aspects of cell biology. Here, we describe one such traditional method, which allows a user to track a molecule of interest within the high-resolution structural context offered by electron microscopy (EM). This method is called pre-embedding immuno-EM (from hereon "pre-embedding") because the immuno-labeling precedes other steps of the EM procedure (Fig. 1) such as specimen embedding in resin, sectioning and ultrastructural analysis under the electron microscope (Baschong and Stierhof, 1998; Humbel et al., 1998; Oliver, 1999).

Of the different EM techniques, pre-embedding is one of the easiest and quickest. It offers a number of advantages over other immuno-EM techniques. First, the labeling step of pre-embedding is almost identical to immunofluorescence staining and does not require any particular skill (Baschong and Stierhof, 1998; Humbel et al., 1998; Polishchuk and Polishchuk, 2013; Polishchuk et al., 2012). Second, pre-embedding can be done with minimal basic EM equipment and does not require specific low temperature devices that are needed for a number of other immuno-EM methods (Brown et al., 2009; Polishchuk and Polishchuk, 2013; Polishchuk et al., 2012). Third, pre-embedding images look like familiar standard EM pictures with the addition of electron dense labeling over the structures of interest. This happens because the pre-embedding protocol involves standard an OsO_4 post-fixation step, which gives high contrast to the membranes, similarly to conventional EM images (Baschong and Stierhof, 1998; Humbel et al., 1998; Oliver, 1999; Polishchuk and Polishchuk, 2013; Polishchuk et al., 2012).

Like other EM methods, pre-embedding has to deal with a compromise between ultrastructural preservation and antigen availability for immuno-labeling. Ultrastructural preservation is achieved by the addition of low amounts of glutaraldehyde to the fixative. Unfortunately, glutaraldehyde destroys the antigenicity of some proteins even at minimal concentrations (Griffiths et al., 1993; Humbel et al., 1998). However, any antigen that maintains its reactivity after the application of EM-specific fixative for pre-embedding can be detected in association with well-preserved subcellular structures (Griffiths et al., 1993; Humbel et al., 1998). Moreover, pre-embedding labeling still offers better accessibility and preservation of antigens compared to post-embedding method, which allows only a very limited number of antigen sights to be exposed to antibody at the surface of plastic section (Baschong and Stierhof, 1998; Melo et al., 2014). As a consequence, only very abundant and stable proteins can be detected

* Corresponding author.
E-mail address: polish@tigem.it (R.S. Polishchuk).

https://doi.org/10.1016/j.tice.2018.09.009
Received 14 May 2018; Received in revised form 19 September 2018; Accepted 25 September 2018
Available online 27 September 2018

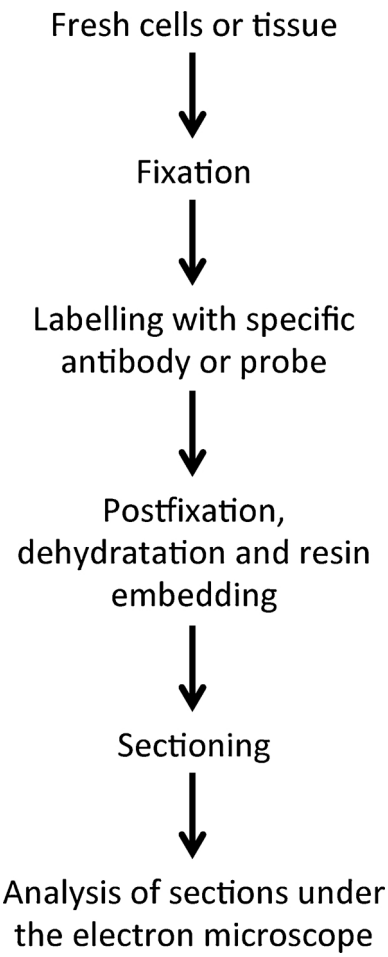

Fig. 1. Schematic workflow of pre-embedding labeling procedure.

with post-embedding strategy (Baschong and Stierhof, 1998; Melo et al., 2014), while pre-embedding does not impose this limitation. It is important to note that the success of pre-embedding also depends on the use of appropriate secondary antibodies. Antibody penetration to the structures in the cell interior needs to be facilitated by permeabilizing cells or tissues with detergents (see below).

Even though 12–15 nm membrane pores are created during permeabilization, some traditional secondary antibodies conjugated with HRP or gold particles of 5–15 nm are still too big to pass through the fixed meshwork of cytoplasmic structures. Therefore, to ensure free penetration into the cell, Fab fragments of secondary antibodies have been combined with either HRP (Brown et al., 1984; Brown and Farquhar, 1984) or ultrasmall (1.4 nm) gold particles (Hainfeld and Furuya, 1992) to ensure the success of the pre-embedding technique. Indeed, 1.4 nm gold particles conjugated only with the Fab fragment of IgG are currently the most popular and widely used option in pre-embedding, thus significantly optimizing labeling efficiency and specificity.

The pre-embedding technique includes a group of strategies each designed for the optimal detection of the antigen of interest. Here, we will make a short excursion into the history of these techniques, try to underscore their advantages and caveats, and share critical know-how that may help to successfully apply pre-embedding in your research. Finally, we will discuss how pre-embedding could be combined with novel advanced EM technologies such as correlative-light electron microscopy (CLEM) or Focused-Ion Beam-Scanning Electron Microscopy (FIB-SEM). Number of emerging pre-embedding procedures exploits specific tags such as APEX2, methallotionein and mini-SOG, but those will be not presented because out of the scope of this review and they have been discussed elsewhere in great details (Martell et al., 2012; Risco et al., 2012; Shi et al., 2017; Shu et al., 2011).

1.1. HRP-based pre-embedding

HRP-based labeling represents the oldest pre-embedding approach. It is based on the ability of horseradish peroxidase (HRP) to generate free oxygen radicals during H_2O_2 hydrolysis. These radicals oxidize the chromogenic substrate 3,3 diaminobenzidine (DAB) into an electron-dense product that can be easily detected under the electron microscope. Several decades ago, this property of HRP was used to observe HRP internalization in cells and tissues and, hence, to reveal the organization and dynamics of endocytic compartments (Steinman et al., 1974).

Later, two strategies were developed to use HRP for pre-embedding labeling. The first strategy is based on the detection of endogenous proteins of interest using the Fab fragment of a secondary antibody conjugated with HRP (Brown et al., 1984; Brown and Farquhar, 1984). The second strategy requires the engineering of a DNA construct that combines the protein of interest with the HRP tag (Connolly et al., 1994; Stinchcombe et al., 1995). The latter approach is particularly useful when the antigenicity of the endogenous protein is easily compromised by glutaraldehyde during cell/tissue fixation. Regardless of which strategy is used, the outcome of HRP/DAB method is the same and consists in the generation of a dark precipitate in the compartment where the protein of interest is located (see examples in Fig. 2).

HRP-based pre-embedding is especially effective for the detection of proteins residing in membrane-enclosed compartments. For this reason it was widely used in the membrane trafficking field to reveal the dynamics of proteins, compartments and entire organelles in the secretory and endocytic pathways. This immuno-HRP approach was employed in several pioneering papers to reveal the localization and trafficking of endogenous mannose-6 phosphate receptor in different cells and tissues (Brown et al., 1984; Brown and Farquhar, 1984) and to define the intracellular routes of several viral proteins (Kuismanen et al., 1982; Saraste and Kuismanen, 1984). Later, it helped to characterize the compartmentalization of glycosylation enzymes in the Golgi apparatus (Kweon et al., 2004) as well as their fate in mitosis (Zaal et al., 1999) and upon microtubule disassembly (Polishchuk et al., 1999). In addition, the nature of post-Golgi carriers and the mechanism of export of constitutive cargo proteins from the TGN were defined with extensive use of the HRP/DAB technique (Polishchuk et al., 2003, 2000). The generation of HRP-tagged constructs significantly expanded the applications of the pre-embedding approach. For example, it helped to follow the trafficking of soluble and trans-membrane proteins to and from the Golgi (Connolly et al., 1994; Stinchcombe et al., 1995) and to understand the mechanisms of de novo Golgi biogenesis (Jollivet et al., 2007).

Alternatively, several HRP-conjugated ligands can be used instead of antibodies to label proteins, lipids, sugars or even specific compartments/pathways of interest. HRP-tagged lectins have been widely used for this purpose (Pavelka et al., 1998; Ranftler et al., 2013). Lectins represent ligands that bind carbohydrates specifically at cellular membranes, so they can be easily internalized by receptor-mediated endocytosis. Thus, their trafficking and localization can be tracked within the cell. For example, wheat germ agglutinin (WGA) lectin labeled with HRP was widely used to show the connection of endocytic compartments with the trans-Golgi network (TGN), thus underlying an important role in maintenance of secretory and endocytic pathways (Pavelka et al., 1998). Another lectin, *Helix Pomatia*, conjugated with HRP was first used to demonstrate its preferential binding to alpha-*N*-acetyl-D-galactosamine (GalNAc) residues and its favored localization to the *cis*-Golgi compartment (Pavelka and Ellinger, 1985). The *Helix Pomatia* lectin then became one of the specific tools for recognizing GalNAc residues and the *cis*-part of the Golgi complex both in immunofluorescence and electron microscopy studies (Pavelka and