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Invited Review

A method of correlative light and electron microscopy for yeast cells

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ABSTRACT

Correlative light and electron microscopy (CLEM) is a method of imaging in which the same specimen is observed by both light microscopy and electron microscopy. Specifically, CLEM compares images obtained by light and electron microscopy and makes a correlation between them. After the advent of fluorescent proteins, CLEM was extended by combining electron microscopy with fluorescence microscopy to enable molecular-specific imaging of subcellular structures with a resolution at the nanometer level. This method is a powerful tool that is used to determine the localization of specific molecules of interest in the context of subcellular structures. Knowledge of the localization of target proteins coupled with the functions of the structures to which they are localized yields valuable information about the molecular functions of these proteins. However, this method has been mostly applied to adherent cells due to technical difficulties in immobilizing non-adherent target cells, such as yeasts, during sample preparation. We have developed a method of CLEM applicable to yeast cells. In this report, we detail this method and present its extension to Live CLEM. The Live CLEM method enabled us to link the dynamic properties of molecules of interest to cellular ultrastructures in the yeast cell. Since yeasts are premier organisms in molecular genetics, combining CLEM with yeast genetics promises to provide important new findings for understanding the molecular basis of the function of cellular structures.

Contents

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1. Introduction

Development of fluorescence microscopy (FM) technologies and the advent of green fluorescent protein (GFP) as a fluorescent probe enabled the determination of the subcellular localization of proteins of interest within a cell. Importantly, it can provide temporal information about a protein when applied to living cells. Thus, it has become a powerful tool for studying the molecular dynamics of cellular structures in a living cell. However, it can only provide relatively low-resolution imaging. In contrast, electron microscopy (EM) provides high-resolution imaging of cellular structures with resolution at the nanometer level. However, it cannot provide temporal information since it cannot be applied to living cells. To overcome these inherent problems, we have developed a method of correlative light and electron microscopy (CLEM) that combines EM with FM (Haraguchi et al., 2008; Kobayashi et al., 2010). This CLEM imaging technology provides temporal and spatial information for specific molecules of interest in the context of cell structures at high-resolution.

In one of the earliest examples of CLEM, interferometry in a light microscope was correlated with electron microscopy (Abandowi and Geissinger, 1975). Development of fluorescent probes, such as GFP (Nakata et al., 1998; Polishchuk et al., 2000; Grabenbauer et al., 2005; Darcy et al., 2006) or bi-functional fluoronanogold (Takizawa and Robinson, 2000), has dramatically improved CLEM analysis. Today one of the most common approaches of CLEM involve the observation of fluorescent probes followed by electron microscopy: the subcellular localization of specific molecules of interest in a cell are first observed using FM and then cellular structures, such as organelles and membranes, in the same cell are observed using EM. Either transmission electron microscopy (TEM) or scanning electron microscopy (SEM) can be used for CLEM. Following image acquisition, FM and EM images are compared to enable the fluorescence images to be correlated with the high-resolution EM images of cellular structures. This method enables analysis of dynamic events involving specific molecules of interest in the context of specific cellular structures. Recently a fluorescent flavoprotein, miniSOG, has been developed as a genetically encoded tag that is applicable for both FM and EM. Like GFP, miniSOG-tagged proteins can be visualized by FM, and miniSOG generates singlet oxygens that convert diaminobenzidine (DAB) into an osmiophillic polymer that can be resolved by EM (Shu et al., 2011). By combining fluorescent proteins that can be observed in living cells with high resolution EM, CLEM provides a powerful tool for examining complex, dynamic biological events, such as nuclear envelope reformation and autophagosome formation, in mammalian cells (Haraguchi et al., 2008; Kobayashi et al., 2010).

To date, due to technical difficulties associated with the movement/drift of non-adherent cells in the medium, particularly during preparation of the sample for EM following the acquisition of FM images, CLEM has usually been applied to the study of adherent cells. We have developed a method involving lectins (sugar-binding proteins) to immobilize yeast cells for CLEM imaging. Here we describe a detailed method of CLEM imaging and its extension to Live CLEM for yeast cells. Combining CLEM imaging with yeast genetics will create a powerful tool which can be used to investigate the function of cellular structures at the molecular level.

2. Materials and methods

2.1. Fluorescence microscope set-up

A DeltaVision microscope system (Applied Precision Inc., Seattle, USA) based on an Olympus wide-field fluorescence microscope IX70 (Olympus Corp., Japan) was used for FM imaging. Fluorescence images were obtained using an interline CoolSNAP HQ^2 CCD camera (Photometrics, USA) as an image detector through an oil-immersion objective lens (PlanApo 60×, NA = 1.4, Olympus). Z-stack images were obtained with this setting and subjected to deconvolution to improve FM images by removing out-of-focus images (Agard et al., 1989). The softWoRx® software equipped with the microscope system was used for deconvolution. A temperature-controlled room was used to keep the temperature of the cells and the microsope at 26 °C during live observation as described previously (Haraguchi et al., 1999).

2.2. Preparation of yeast cells

2.2.1. Cultivation of yeast cells

In this study, the fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae* harboring GFP-fusion genes of interest were used. The cells of interest were picked from a single colony and cultured in an appropriate culture medium for vegetative growth: Typically, YES medium and YPD medium were used for *S. pombe* and *S. cerevisiae*, respectively. For live-cell imaging of growing cells, *S. pombe* cells (*S. cerevisiae* was not used for live-CLEM) were collected by centrifugation at 3000 rpm (1200 × *g*) for 5 min, suspended in an optically clear and colorless medium (*e.g.* filtered EMM2 medium), and then incubated in the medium overnight before observation. For live-cell imaging of meiotic cells, *S. pombe* cells were first induced to undergo meiosis by culture on an agar plate containing ME medium, then collected and suspended in EMM2-N medium (an optically clear and colorless medium), and immediately immobilized on the coverslip of a glass-bottom dish (see Section 2.2.2). The compositions of culture media can be found in the literature (Sherman et al., 1983; Moreno et al., 1991).

2.2.2. Coating of the surface of coverslip for immobilization of the cells

Identification of specific cells of interest is required for CLEM imaging. For this purpose, we used a gridded glass-bottom dish (a plastic culture dish with a gridded coverslip, instead of a regular coverslip, attached to the opening in the bottom of the dish) (P/N 81148 and 81168, ibidi, Germany) (Fig. 1A and B), and coated the surface of the coverslip with lectins to immobilize the yeast cells.