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Review

The intracellular trafficking pathway of transferrin ☆

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ABSTRACT

Background: Transferrin (Tf) is an iron-binding protein that facilitates iron-uptake in cells. Iron-loaded Tf first binds to the Tf receptor (TfR) and enters the cell through clathrin-mediated endocytosis. Inside the cell, Tf is trafficked to early endosomes, delivers iron, and then is subsequently directed to recycling endosomes to be taken back to the cell surface.
Scope of review: We aim to review the various methods and techniques that researchers have employed for elucidating the Tf trafficking pathway and the cell-machinery components involved. These experimental methods can be categorized as microscopy, radioactivity, and surface plasmon resonance (SPR).
Major conclusions: Qualitative experiments, such as total internal reflectance fluorescence (TIRF), electron, laser-scanning confocal, and spinning-disk confocal microscopy, have been utilized to determine the roles of key components in the Tf trafficking pathway. These techniques allow temporal resolution and are useful for imaging Tf endocytosis and recycling, which occur on the order of seconds to minutes. Additionally, radiolabeling and SPR methods, when combined with mathematical modeling, have enabled researchers to estimate quantitative kinetic parameters and equilibrium constants associated with Tf binding and trafficking.
General significance: Both qualitative and quantitative data can be used to analyze the Tf trafficking pathway. The valuable information that is obtained about the Tf trafficking pathway can then be combined with mathematical models to identify design criteria to improve the ability of Tf to deliver anticancer drugs. This article is part of a Special Issue entitled Transferrins: Molecular mechanisms of iron transport and disorders.

1. Introduction

Transferrin (Tf) is an iron-binding protein that facilitates iron-uptake in cells. Iron-loaded Tf, also known as holo-Tf, binds to the Tf receptor (TfR) and enters the cell through clathrin-mediated endocytosis [1]. Two types of Tf receptors exist, TfR1 and TfR2 [2]. TfR2 is a close homologue of TfR1 showing 45% identity in the extracellular domain [3]. TfR2 is predominantly found in tissues responsible for regulating iron metabolism such as the liver and small intestine whereas TfR1 distribution is less restricted [4–5]. TfR2 has an approximately 25-fold reduced holo-Tf affinity compared to TfR1 and is believed to function in overall iron metabolism in addition to cellular iron uptake [6]. In this review, we focus mainly on TfR1 which will be referred to hereafter as simply TfR.

When ligands, such as holo-Tf, are internalized into the cell through clathrin-mediated endocytosis, as shown in Fig. 1, endocytosed ligands are sorted along the trafficking pathway into three main populations of endosomes: early endosomes, late endosomes, and recycling endosomes [7]. Studies suggest that ligand sorting begins at the cell surface, separating ligands into two distinct types of early endosomes with different maturation kinetics: dynamic or static [8–9]. Early endosomes maturing quickly into late endosomes comprise the dynamic population, whereas the slower-maturing endosomes comprise the static population. Ligands destined for degradation, such as low-density lipoprotein (LDL), have been shown to be preferentially trafficked to the dynamic population of early endosomes [8]. Tf, on the other hand, follows the recycling pathway and indiscriminately enters both populations of early endosomes [8]. However, since the population of static early endosomes is much greater than that of the dynamic early endosome, Tf becomes enriched in the static population of early endosomes. This pre-early endosomal sorting process is believed to be the first step in the segregation of cargo destined for degradation from those intended to be recycled back to the cell surface.

Both populations of early endosomes undergo a second sorting process to direct cargo intended for recycling to the recycling pathway. This sorting process is initiated with the development of tubular formations in early endosomes, which eventually separate and shuttle cargo either directly back to the cell surface or to the perinuclear endocytic recycling compartment [10]. These two different recycling routes differ in time, since routing through recycling endosomes takes longer than directly being trafficked to the cell surface from the early endosomes [8, 11]. Since Tf is contained within both dynamic and static early endosomes, these tubular formations function to traffic Tf to the recycling pathways from both populations of early endosomes. Ligands intended for degradation remain in the early endosomes, which then mature into late

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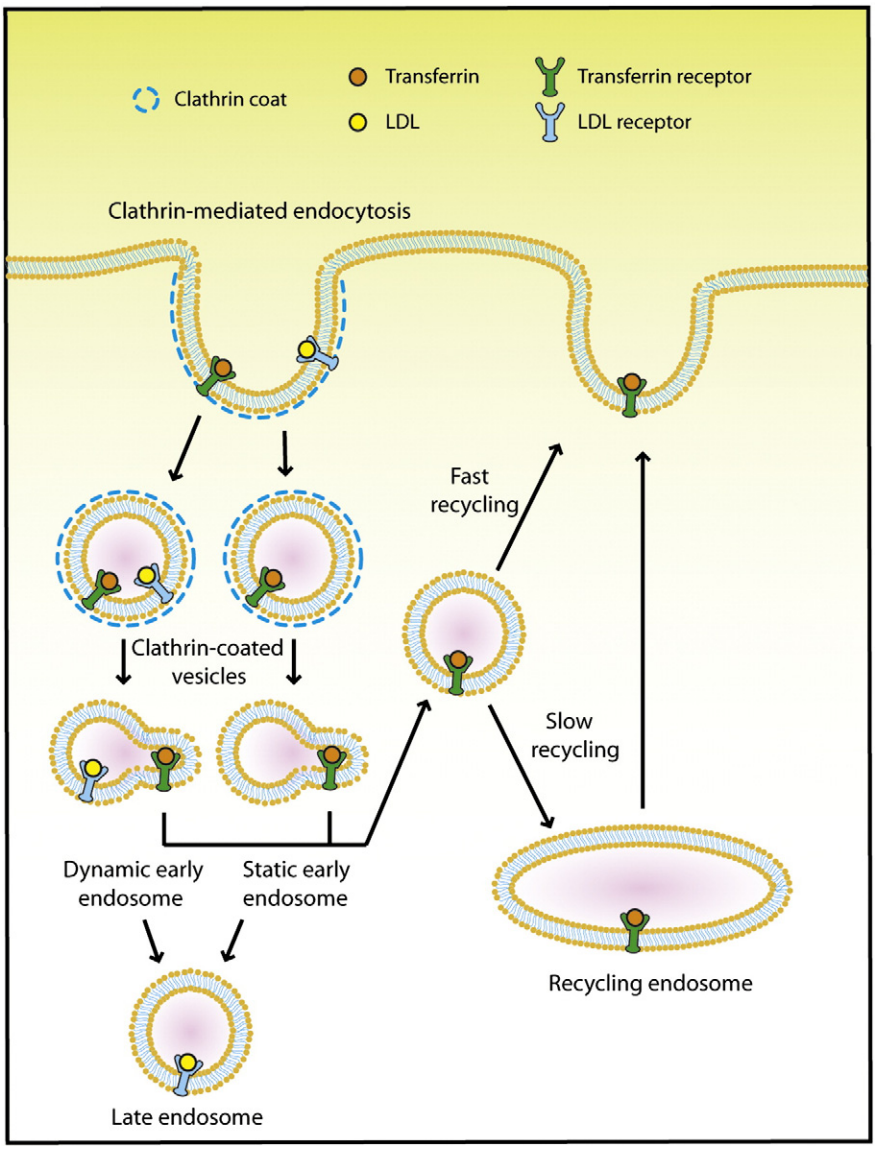

Fig. 1. An overall diagram of Tf trafficking. Tf is internalized via clathrin-mediated endocytosis and taken up into clathrin-coated vesicles. After the uncoating of clathrin, Tf can be found in both populations of static and dynamic early endosomes, as Tf shows no preference for either population. LDL, which follows the degradation pathway, is trafficked preferentially to dynamic early endosomes. Tubulation occurs in both populations of early endosomes which proceeds to separate from early endosomes and traffic Tf to recycling pathways. A fast recycling route takes Tf directly back to the plasma membrane. A slower recycling route delivers Tf first to the perinuclear recycling compartment before Tf is trafficked back to the cell surface. For more information, reviews by Conner and Schmid [13] and Grant and Donaldson [14] describe additional information in further detail on clathrin-mediated endocytosis and recycling pathways, respectively.

endosomes that subsequently proceed toward the degradation pathway involving lysosomes [12]. An overall diagram of Tf trafficking is shown in Fig. 1. For more detailed diagrams of the Tf trafficking pathway, please see reviews by Conner and Schmid [13] and Grant and Donaldson [14], which describe the clathrin-mediated endocytosis and recycling pathways, respectively.

Tf internalization via clathrin-mediated endocytosis and subsequent intracellular trafficking through recycling pathways have been extensively studied. Here, we aim to review the cell-machinery components involved, as well as the methods and techniques that researchers have employed for elucidating the Tf trafficking pathway. These experimental methods can be categorized as microscopy, radioactivity, and surface plasmon resonance (SPR). Microscopy provides a qualitative assessment for determining which cellular machinery-components are involved and the intracellular locations where they function. Radioactivity and SPR, on the other hand, are primarily used in conjunction with mathematical models to determine quantitative information regarding the kinetics of Tf binding, internalization, and recycling. This quantitative information can then be used in mathematical models to identify design criteria for improving the drug delivery efficacy of Tf. Thus, we have structured this review into two main parts. The first part focuses on microscopy experiments that have been performed to elucidate which cellular-components are involved in Tf intracellular trafficking and their locations of action. The second part focuses on radioactivity and SPR experiments, as well as the corresponding mathematical models, which allow us to estimate rate constants for different steps in the intracellular trafficking pathway.

2. Investigating the Tf trafficking pathway using microscopy

2.1. Clathrin-mediated endocytosis

The internalization of Tf into the cell begins with clathrin-mediated endocytosis, where the following core machinery-components are involved: clathrin, adaptor proteins, and dynamin [13, 15–17]. The clathrin complex consists of three heavy and three light chains which