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Visualizing molecular diffusion through passive permeability barriers in cells: conventional and novel approaches

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Diffusion barriers are universal solutions for cells to achieve distinct organizations, compositions, and activities within a limited space. The influence of diffusion barriers on the spatiotemporal dynamics of signaling molecules often determines cellular physiology and functions. Over the years, the passive permeability barriers in various subcellular locales have been characterized using elaborate analytical techniques. In this review, we will summarize the current state of knowledge on the various passive permeability barriers present in mammalian cells. We will conclude with a description of several conventional techniques and one new approach based on chemically inducible diffusion trap (CIDT) for probing permeable barriers.

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Introduction

Diffusion is the random motion of molecules driven by thermal energy, resulting in molecular movement from areas of high concentration to areas of low concentration. As an energetically favorable process, diffusion occurs without energy expenditure and thus is a ubiquitous strategy used by cells for molecular transport. However, uncontrolled diffusion can be disadvantageous to achieving localized signaling, which often requires the spatial enrichment of signaling species and is critical to fundamental cellular functions such as cell polarity, growth, proliferation, and death [1•,2–4,5••]. To address this issue, cells have evolved diffusion barriers, which serve as gatekeepers in filtering molecules based on size, shape, charge, and other intrinsic properties. Diffusion barriers thus enable cellular compartmentalization and spatiotemporal control of signaling. Intracellular diffusion barriers exist at a variety of cellular structures, including the nuclear envelope, the annulus of spermatozoa, the leading edge of migrating cells, the cleavage furrow of dividing cells, and the budding neck of yeast, as well as in cellular extensions such as primary cilia, dendritic spines, and the initial segment of the neuronal axon [1•,6•,7]. While some diffusion barriers exist constitutively in cells, others are highly dynamic. The importance of diffusion barriers is further underscored by the various human diseases which result from their dysfunction [3,6•,8,9].

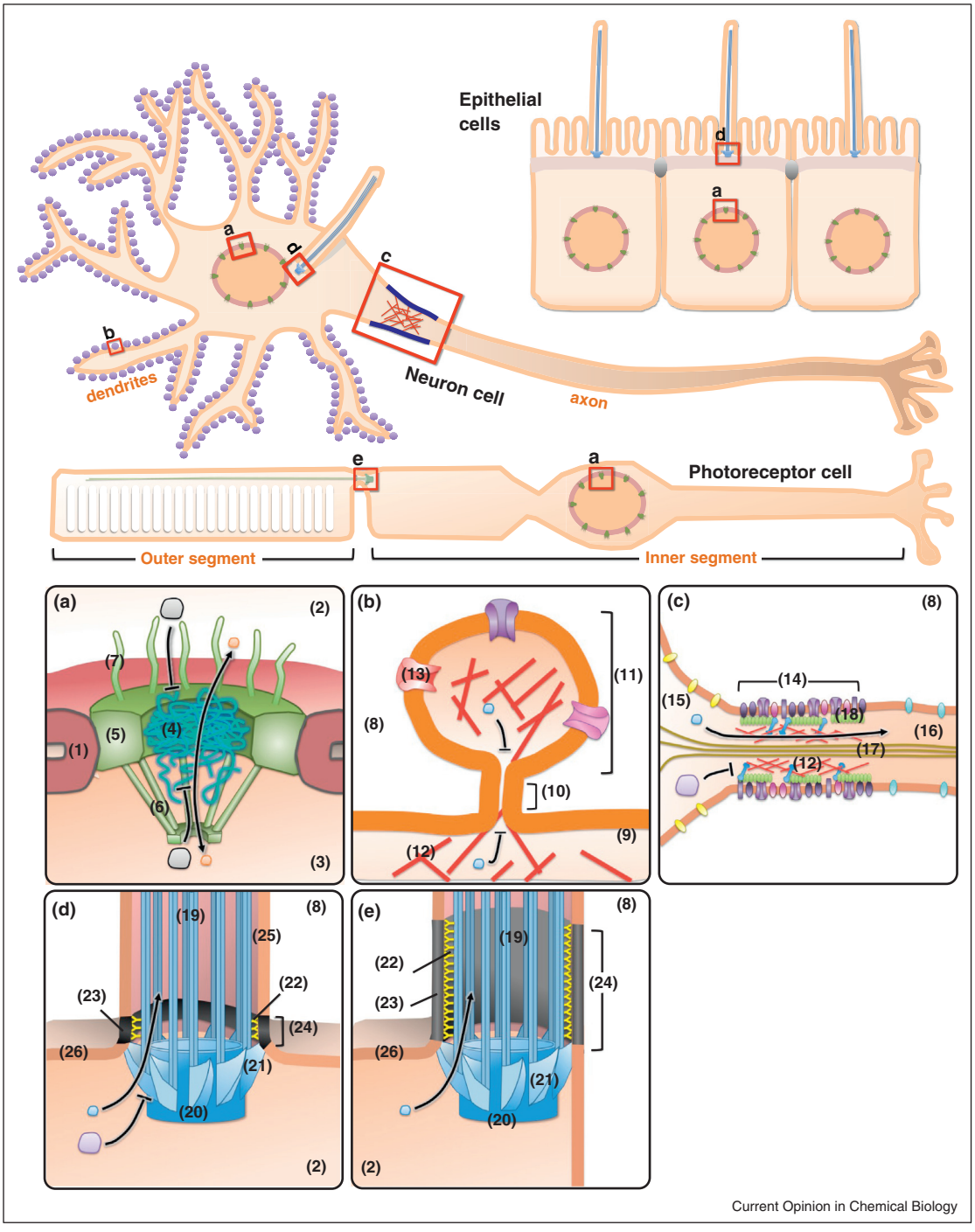
Diffusion barriers can be categorized into two major classes based on the substrates they affect: lateral diffusion barriers and permeability barriers. Lateral diffusion barriers localize in membranes and restrict the movement of molecules within the membrane plane such as transmembrane proteins and membrane lipids [1•]. Conversely, permeability barriers embedded within membranes act as conduits regulating the movement of solutes through the membrane. Permeability barriers also localize within aqueous cellular compartments to hinder solute diffusion [2,10••]. Generally speaking, lateral diffusion barriers are well characterized [1•], primarily because membrane molecules move relatively slowly and are in a two-dimension environment, allowing easy observation of their dynamics. In contrast, it has been challenging to measure the dynamics of solutes in cells, owing to their generally fast diffusion as well as technical limitations in precisely determining the axial position of solute molecules inside living cells [10••]. However, recent advances in microscopy techniques have enabled the refinement of our understanding of passive permeability barriers. In this review, we will provide an overview of the current knowledge of permeable diffusion barriers in various subcellular regions (summarized in Figure 1 and Table 1). Subsequently, we will describe six methods used to measure the dynamics of solutes in cellular aqueous compartments and their application to probing permeable diffusion barriers, with a particular focus on the strengths and weaknesses of these approaches (summarized in Figure 2 and Table 2).

Passive permeability barriers in cells

Nuclear pore complex

The eukaryotic nucleus is surrounded by the nuclear envelope, a double layered membrane structure that functionally separates the nucleus from the cytosol. Communication between the cytosol and nucleus is regulated by specialized conduits, known as nuclear pore complexes (NPCs), which are anchored in the nuclear envelope at

Figure 1



Passive permeability barriers in cells. Cellular structures that harbor passive permeability barriers are represented by **(a)** nuclear pore complex, **(b)** dendritic spines, **(c)** axon initial segment, **(d)** primary cilium, and **(e)** connecting cilium. The detailed structures are denoted by Arabic numbers: (1) nuclear envelope, (2) cytoplasm, (3) nucleoplasm, (4) central tube, (5) scaffold ring, (6) nuclear basket, (7) cytoplasmic filament, (8) extracellular space, (9) dendrite, (10) spine neck, (11) spine head, (12) actin filament, (13) spine receptors, (14) axon initial segment, (15) somatodendritic compartment, (16) axon, (17) microtubules, (18) ankyrin-G proteins, (19) axoneme, (20) basal body, (21) transition fibers, (22) Y-shaped linkers, (23) ciliary necklace, (24) transition zone, (25) ciliary membrane and (26) plasma membrane.