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Review

A broad view of scaffolding suggests that scaffolding proteins can actively control regulation and signaling of multienzyme complexes through allostery ☆

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ABSTRACT

Enzymes often work sequentially in pathways; and consecutive reaction steps are typically carried out by molecules associated in the same multienzyme complex. Localization confines the enzymes; anchors them; increases the effective concentration of substrates and products; and shortens pathway timescales; however, it does not explain enzyme coordination or pathway branching. Here, we distinguish between metabolic and signaling multienzyme complexes. We argue for a central role of scaffolding proteins in regulating multienzyme complexes signaling and suggest that metabolic multienzyme complexes are less dependent on scaffolding because they undergo conformational control through direct subunit–subunit contacts. In particular, we propose that scaffolding proteins have an essential function in controlling branching in signaling pathways. This new broadened definition of scaffolding proteins goes beyond cases such as the classic yeast mitogen-activated protein kinase Ste5 and encompasses proteins such as E3 ligases which lack active sites and work via allostery. With this definition, we classify the mechanisms of multienzyme complexes based on whether the substrates are transferred through the involvement of scaffolding proteins, and outline the functional merits to metabolic or signaling pathways. Overall, while co-localization topography helps multistep pathways non-specifically, allosteric regulation requires precise multienzyme organization and interactions and works via population shift, either through direct enzyme subunit–subunit interactions or through active involvement of scaffolding proteins. This article is part of a Special Issue entitled: The emerging dynamic view of proteins: Protein plasticity in allostery, evolution and self-assembly.

1. Introduction

How are enzymatic actions in signaling pathways, and in metabolism, coordinated and controlled? Here, we focus on the important role of allostery in controlling multienzyme complexes and contrast allosteric mechanisms to other ways of regulation, such as subcellular co-compartmentalization topography. Our central thesis is that scaffolding proteins are central to the regulation of signaling multienzyme complexes. In contrast, metabolic multienzyme complexes are less dependent on scaffolding because they undergo more direct conformational control through subunit–subunit contacts. We assign scaffolding proteins a much more active, fine-tuning role than considered to date, one that actively involves conformational regulation through allostery. The yeast mitogen-activated protein kinase Ste5 can be viewed as a classic scaffold protein. Our view of scaffolding proteins is broader. We adopt a functional definition of scaffolding that also involves proteins such as E3 ligases, which have not been considered as scaffolding to date; but which work via allostery to facilitate the ubiquitin transfer reaction from the E2 enzyme to the substrate protein. Further, scaffolding proteins not only bias the conformational ensembles locally; via the cytoskeleton network, it can bias the ensembles across the cell.

This new viewpoint is important because it designates scaffolding proteins as *active* key players in signaling pathways where multienzyme complexes invariably control pathway switching, often via post-translational modifications.

2. An overview: co-localization cannot provide effective answers to multienzyme regulation

In large part, enzymes do not function autonomously [1,2]; instead they are integral elements of signaling or biochemical (metabolic) pathways, where a product of one reaction can serve as the substrate or

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precursor for the next, with the end products achieved through multiple chemical steps (Fig. 1). Sequential enzymatic pathway steps require that enzymes be confined, caged in proximity to each other. A shared localization fastened at a specific cellular environment is of crucial importance: the enzymes are juxtapositioned proximal to the prior and following stages in the cellular network. Such an organization integrates the enzymes into the global cell responses to the environment. Co-localization also increases the effective local concentration of substrates/products and avoids the otherwise long-range diffusion–collision of the enzymes and their substrates. In signaling pathways, it makes possible an orchestrated translation of a signal into distinct events, particularly phosphorylation and ubiquitination. Related enzymatic steps are mainly carried out by multienzyme complexes which are anchored at a specific cellular sub-compartmentalization. A multienzyme complex can consist of multiple domains in a single polypeptide chain, distinct subunits, or both, that possess more than one catalytic site. The efficiency of the complex can be particularly high if the active sites of consecutive steps communicate directly, mediating the transfer of a molecule. Key questions include how are consecutive enzymatic steps accommodated? How can multienzyme pathways branch, and how is branching controlled? Why metabolic multienzyme complexes typically do not contain scaffolding proteins while signaling complexes do? On its own, co-localization is not able to provide effective answers. Here we propose and provide supporting data from the literature and our own work that allostery can play key roles in (*i*) controlling successive enzymatic steps in multienzyme complexes, and (*ii*) that it does so either via direct contact of sequestered, proximal enzymes, or via scaffolding proteins. We suggest (*iii*) that while in metabolic enzyme pathways sequestration and localization may be key elements this is not the case for multienzyme complexes in signaling pathways. *In particular, we argue* (*iv*) *that scaffolding is much more prevalent than has been previously assumed; and enzyme domains, other enzymes, and other proteins to which the enzymes or the substrates attach, can also fulfill this role*. Scaffolding facilitates effective control and switches which help in determining pathway branching; at the same time, it also allows combinatorial assembly of enzyme components, which expands their functional diversity.

3. Contrasting allosteric mechanisms to other ways of regulation of multienzyme complexes such as subcellular co-compartmentalization

Allostery is a key regulator of protein activity; and thus of pathways and cell function [3–9]. Allostery is a cooperative event, linking perturbations at one (allosteric) site with their consequences at another (the active or binding site). Allosteric outcomes can be expressed by a larger enzyme population bearing distinct changes in the active site shape or dynamics [10–13]. The changes at the active site specify ligand selection and catalytic action [9]. Allostery can control function not only by changing the local shape (or dynamics) of the active site; but also by biasing the sampling of the three-dimensional space by the domains bearing the active sites [7,14] which are responsible for sequential enzymatic steps. These can be in the same or in different molecules in the multienzyme complex. Alternatively, allostery can work via a conformational change of these domains (Fig. 2). The importance of allosteric modulation in multienzyme complexes can hardly be over-emphasized, when we consider transfer of products/substrates along enzymatic pathways. The allosteric modulators can be the substrate or product; a cofactor, protein, or a second messenger; and the modulation can take place via non-covalent or covalent binding events, such as phosphorylation, ubiquitination and neddylation [15]. The outcome can be a rotation, bringing the reactive, active site-linked substrate closer to its target, followed by transfer of the substrate from the catalytic site to the target. Such action can be mediated by an enzyme which works by creating a favorable environment, as in the case of ubiquitin transfer from E2 to the target in the cullin RING ligases [16]. Alternatively, conformationally-biased fluctuation of a highly flexible linker can also result in similar outcome. For allostery to be at play, precise physical interactions between the enzymes, or the enzymes and the scaffolding protein, are a prerequisite. Mutations at the protein–protein interface or far away can impede allosteric control. Similarly, mutations affecting allosteric propagation routes can obstruct allosteric regulation. The interactions between the enzymes can be short-lived; however, they should be for a sufficiently long time for the allosteric signal to go through.

Over the years, allostery has been described as the linkage between two sites in the structure, with the allosteric event far away defining substrate specificity via the active site conformation and dynamics, and binding affinity. Multienzyme complexes show that the conformation and local fluctuations at the active site may not be the sole factor; the allosterically-governed positioning of the site, or of the domain bearing the site, can also play a key role on a global scale. Allostery can work by cooperatively enhancing the tendency of the site to re-orient in a productive direction [7]. This can facilitate transfer of substrates along enzymatic pathways in multienzyme complexes; it also poses the challenge of discovery of allosteric drugs that modulate function by biasing domain rotations.

Collectively, here we propose that co-localization (or sub-compartmentalization) on its own is unlikely to coordinate and control multienzyme function. Allosteric action, either directly between enzymes, or via scaffolding proteins, are key factors in

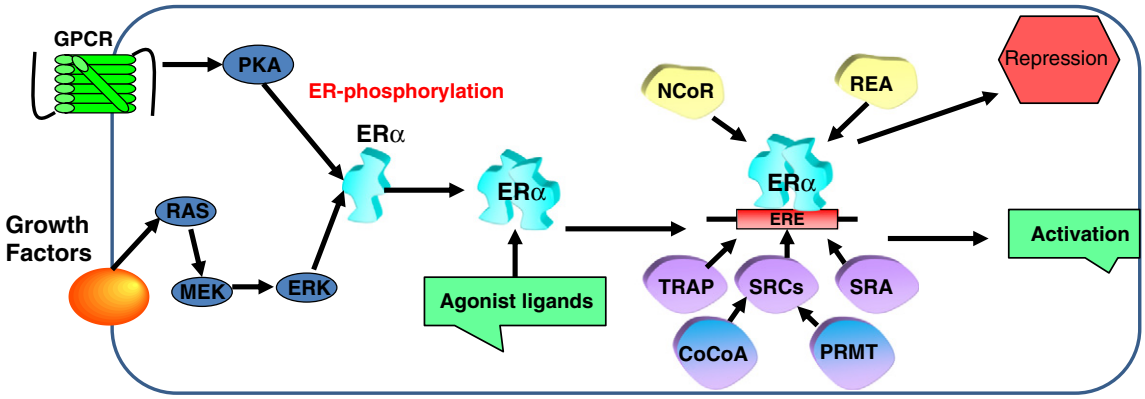

Fig. 1. An outline of estrogen receptor (ER) signaling as an example of the multiple chemical steps in signaling pathways. Estrogen receptors (Erα and ERβ) can be selectively activated by extracellular signals, hormone and co-factor binding events [89], and phosphorylation of the ER monomer. Examples of the extracellular signals are: (i) binding of dopamine and cAMP to GPCR can activate PKA; (ii) growth factors (GFs) activate their receptors with subsequent activation of the RAS–RAF–ERK pathway. Cofactors [90] like the nuclear receptor corepressor (NCoR) and the repressor of the estrogen receptor activity (REA) lead to repression of ER response elements (ERE). Examples of direct activators are the thyroid hormone receptor (TRAP), steroid receptor activator (SRA), and steroid receptor co-activators (SRCs). Secondary co-activators (like CoCoA and PRMT) also bind ERS indirectly through association with SRCs.