Regulated intracellular association of proteins using cell-permeable synthetic ligands

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Chemistry & Biology 15 April 1994, Introductory issue

Nature maintains homeostasis in living systems through remarkable control mechanisms that are only now being elucidated. These mechanisms frequently involve cascades of regulated dimerization or oligomerization of proteins. For example, extracellular factors cause homo- or heterodimerization of cell-surface receptors. This process results in the dimerization of the intracellular domains of the receptors and the subsequent recruitment of intracellular signalling proteins. Depending on the identity of these proteins, the signal transduced across the membrane may result in cell proliferation, differentiation, cell cycle arrest, or death. Later in the signalling cascade, signals that reach the nucleus can induce transcription factors to interact with each other and with components of the transcriptional apparatus.

Waves of induced and contingent protein interactions are often seen in biological systems; for example, the gene products expressed in response to the nuclear signals can be additional cell surface receptors and their dimerization-inducing ligands. Signals that reach the cytoplasm can induce cytoskeletal proteins to interact and can induce vesicle fusion by causing a vesicle membrane protein to heterodimerize with a plasma membrane protein. Even the lifetime of cellular proteins can be determined by inducible protein interactions. For example, the human papilloma virus protein E6 appears to target certain cellular proteins for degradation by simultaneously binding to them and to a component of the ubiquitin-dependent proteolytic apparatus.

An example of inducible and contingent protein association is seen in T cell activation. The T-cell receptor (TCR) is associated with a complex of proteins, CD3, which includes the ζ chain. Oligomerization of this complex by an antigen-presenting cell increases the local concentration of kinases associating with the ζ chain. These kinases phosphorylate the ζ chain on tyrosine residues, thus enabling it to bind a cytoplasmic tyrosine kinase that mediates the next step in the signalling cascade.

Our two laboratories have been engaged in a collaboration during the past two years aimed at creating a general solution to controlling intracellular protein association. The goal is to regulate protein–protein interactions simply by treating cells (or animals) with cell-permeable organic molecules. To achieve this goal, we have developed several dimerization domains that can be attached to target proteins for expression in cells as fusion proteins. Symmetric HOD (homo-dimerization) and dissymmetric HED (heterodimerization) reagents induce homo- or heterodimerization of the target protein, respectively. In a recent test of this concept, we used an immunophilin, FKBP12, as a dimerization domain and a dimer of FK506, termed FK1012, as a HOD reagent [1]. FK506 and cyclosporin A are natural products that inhibit signal transduction pathways by forming complexes with immunophilin receptors that block the activity of calcineurin, a part of the TCR signalling pathway (see [2] for review). This activity is irrelevant to the dimerization system we have designed, since the complex of dimeric FK1012 with FKBP12 does not bind to calcineurin. Synthetic chemists have investigated many aspects of FK506 and have even achieved total syntheses of the molecule [2].

In this first example, we attached three FKBP12 domains to a fragment of the ζ chain, which lacked the transmembrane and extracellular domains but was myristoylated to allow association with the cell membrane (see Fig. 1). The ζ chain fusion protein oligomerized in response to the cell permeable FK1012 reagent, triggering the entire cascade of events that normally follow TCR/CD3 aggregation and leading to the transcription of a reporter gene. A monomeric version of FK1012 causes deaggregation of the artificial receptor and termination of the signal. Thus, the system allows both positive and negative control of signalling pathways with cell-permeable, synthetic ligands.

It may be possible to use regulated dimerization of proteins with cell permeable ligands to control a wide variety of cellular processes. Even this first example reveals a useful theme of chemistry-based approaches to biology — synthetic chemistry can provide reagents that are cell permeable and thus well-suited for exploring complex and often mysterious pathways in suitably engineered cells, plants and animals. With this marriage of chemistry and biology, multiple layers of switches can be envisioned, each with its own dimerizing agent, allowing a continuum from molecular to cellular and ultimately to physiological investigations.

References

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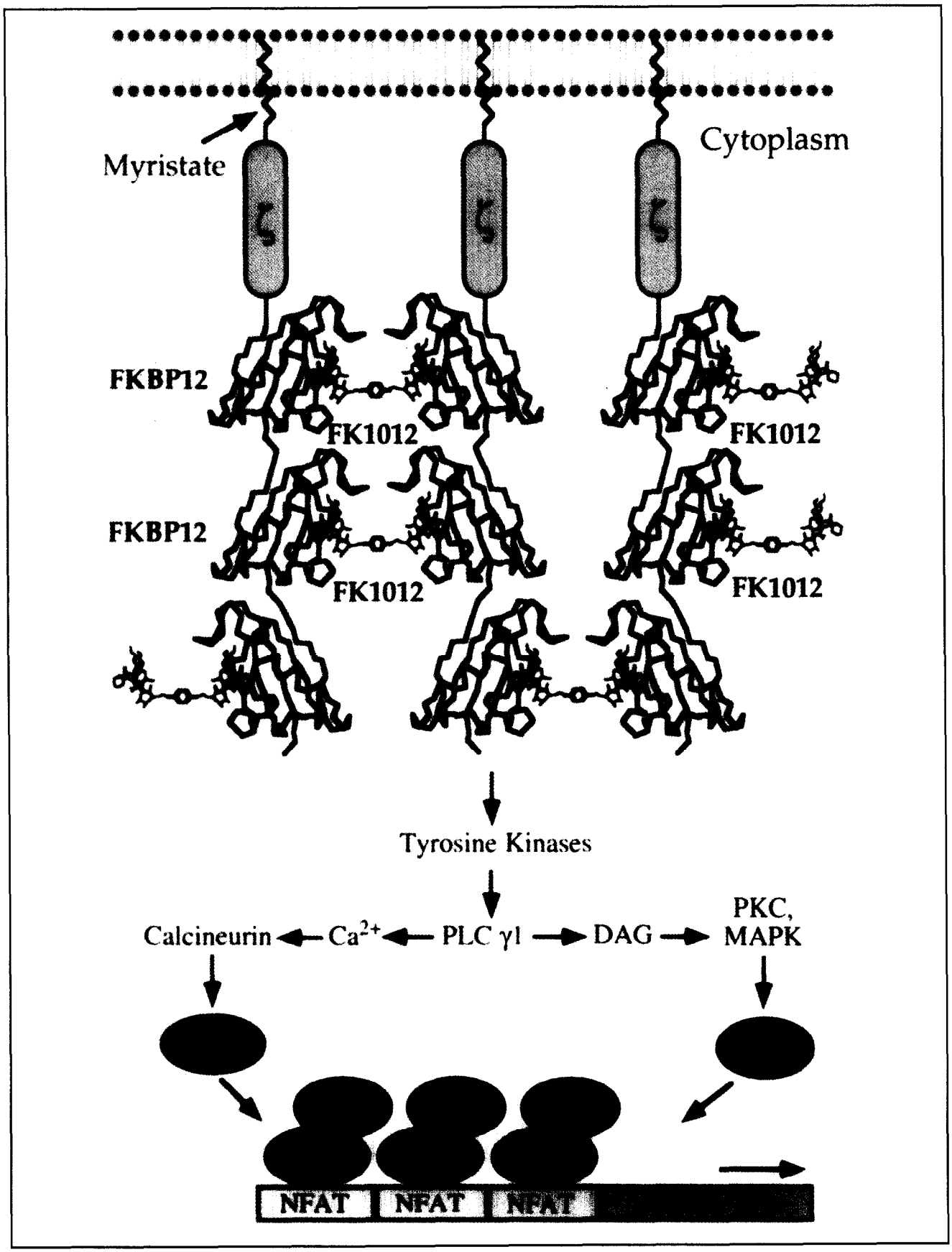

Fig. 1. Oligomerization of ζ via FK506 binding protein 12 (FKBP12) domains induces signalling via the nuclear factor of activated T cells (NFAT). Three FKBP12 domains were attached to a myristoylated fragment of the ζ chain of the T cell receptor, which associates with the plasma membrane. Addition of dimeric FK506 (FK1012) crosslinks the FKBP12 domains, oligomerizing the ζ chains and initiating signalling via ζ-associated tyrosine kinases. PLC-γ, phospholipase C-γ; PKC, protein kinase C; MAPK, mitogen activated protein kinase; NFATc, NFAT cytoplasmic domain; NFATn, NFAT nuclear domain. NFAT binding sites are shown in yellow.