DOCUMENT SUMMARY

This document is a foundational scientific minireview from the journal *Cell*, titled "Translocation and Reversible Localization of Signaling Proteins." It explains the modern "softwired signaling concept," where the movement (translocation) of proteins within a cell is a key part of signal transmission. The paper details how this process is driven by diffusion and reversible binding, how it can be studied using tools like GFP, and the implications for understanding localized and dynamic cellular communication.

FILENAME

research_review_article_signal_transduction_protein_translocation_2000

METADATA

- **Primary Category**: RESEARCH
- **Document Type**: review_article
- **Relevance**: Reference
- **Update Frequency**: Static
- **Tags**: #signal-transduction, #protein-translocation, #cell-biology, #gfp, #diffusion, #signaling-pathways, #neuroscience-foundation, #meyer-lab
- **Related Docs**: This paper provides foundational cellular mechanisms that underpin concepts discussed in "The Neuroscience of Autism."

FORMATTED CONTENT

Translocation and Reversible Localization of Signaling Proteins: A Dynamic Future for Signal Transduction

Mary N. Teruel and Tobias Meyer

Department of Molecular Pharmacology, Stanford University Medical School

An increasing amount of experimental evidence has accumulated over the last years suggesting that most **signal transduction** processes utilize colocalization of sequentially acting signaling proteins for the selective activation of downstream functions (for reviews, see Pawson and Scott, 1997; Tsunoda et al., 1997). In many cases, the activation of signaling proteins by upstream activators or their activation of downstream effectors has been shown to involve binding interactions with adaptor complexes, cytoskeletal structures, and subcellular membranes or with the targets and activators themselves.

> These localization mechanisms can be highly regulated and rapidly reversible, leading to a dynamic view of signal transduction that diverges from the historic **"hardwired signaling concept"** where receptors and other signaling proteins stay largely in place and spatial signal transmission is made possible by the rapid diffusion of second messengers. In contrast, the current **"softwired signaling concept"** is built on the idea that signaling

proteins translocate and undergo reversible binding interactions as key steps of the signal transmission process.

The questions are then raised, whether **translocation** is an active or passive process, how fast it can be, and which type of mechanisms can regulate the dynamic organization of large numbers of signaling proteins in space and time.

The current evidence for this dynamic model of signal transduction stems from immunofluorescence, cell fractionation and related biochemical approaches which have allowed in many cases to obtain snapshots of the changes in the subcellular localization of signaling proteins. For example, the recruitment of cytosolic **SH2 domain** containing proteins by phosphorylated tyrosine residues at the plasma membrane or the nuclear translocation of many cytosolic signaling proteins and transcription factors has been extensively explored by such methods.

Until recently the translocation of signaling proteins could only be followed "live" in cells in selective cases where signaling proteins could be fluorescently labeled in vitro and microinjected into cells for experimentation (Harootunian et al., 1993; Gough and Taylor, 1993). The discovery that **GFP** and its variants can be used as expressed fluorescence tags for signaling proteins and signaling domains led to a new strategy to track the spatio-temporal dynamics of signaling processes by analyzing stimulus-induced changes in their subcellular localization (translocation and colocalization analysis).

A Model for Translocation Based on Diffusion and Reversible Binding Interactions

The investigation of fluorescently conjugated proteins by different microscopy techniques has led to new insights into the dynamic mechanisms of signaling processes. In nearly all cases studied, a significant fraction of cytosolic, membrane-bound or transmembrane proteins diffuse relatively freely within the cytosol or membrane. **Fluorescence recovery after photobleaching (FRAP)** studies of plasma membrane receptors, as well as of membrane-bound and cytosolic signaling proteins, have shown that the **diffusion coefficients** of proteins are quite variable and can be up to 0.05 $\mu m^2/s$ for transmembrane receptors, up to 0.5 $\mu m^2/s$ for membrane-bound proteins and higher than 10 $\mu m^2/s$ for cytosolic proteins.

Given these considerations, how can one interpret an observed subcellular change in the localization of a GFP conjugated signaling protein? A rough analysis of currently reported translocation time courses suggests that nearly all of them are consistent with a random **diffusion** process and are likely triggered by the encounter of a diffusing signaling protein with a newly enabled localized binding site. Over time, the diffusing signaling protein will screen a large region of a cell or membrane for the presence of potential binding partners, giving rise to the observed local enrichment or translocation. Since cellular binding interactions with affinities in the tens of nanomolar to micromolar range are typically rapidly reversible, individual proteins at these target sites can still

dynamically exchange and find different subcellular sites until the system is in a steady state.

Molecular Mechanisms for Protein Translocation

Translocation events in signal transduction are thought to be driven by one of two processes: (1) by the generation of new protein-protein binding interactions or (2) by second messenger-mediated binding interactions between signaling proteins and lipid partners in the plasma membrane or other subcellular structures. Some of the best studied examples in the first category are the generation of GTP-bound small **GTPases** which can lead to the diffusion-mediated recruitment of kinases and other enzymes to the plasma membrane or other subcellular structures. Furthermore, the role of **tyrosine phosphorylation** is often to trigger the recruitment of **SH2 domain-containing proteins** to the plasma membrane, while the phosphorylation of G protein-coupled receptors at serine/threonine residues can lead to the recruitment of arrestin and its isoforms. Some of the best studied examples in the second category include the **Ca^{2+}-**, **phosphoinositide-**, or **diacylglycerol-mediated** plasma membrane translocation of proteins with **C2**, **PH**, or **C1 domains**.

Thus, by utilizing second messenger binding, phosphorylation, GDP to GTP exchange, conformational changes, and other signaling mechanisms, cells can rapidly and reversibly induce new binding sites for signaling proteins and thereby change the localization state of a large class of continuously diffusing signaling proteins.

Subcellular Localization of Signaling Processes

Polar cells such as epithelial cells and neurons, chemotactic cells such as macrophages and fibroblasts, as well as many other cell types, have a need to restrict signaling processes to a small subregion of the cell. This raises the question of how cells can prevent diffusion and translocation processes which would spread the signaling response across the entire cell.

In some signaling pathways, high-affinity cytoskeletal attached adaptor proteins are likely employed to nearly irreversibly localize the signaling proteins within a pathway and thereby prevent their diffusion and translocation. In the case of chemotaxis and in many other local signaling systems, it becomes critical to know how one can estimate the distances over which activated signaling proteins can transmit information. If local activation of a cytosolic, membrane-bound or transmembrane signaling protein occurs, the average distance that the signaling protein travels before it is inactivated can be defined as a "**range of action (r)**" dependent on the diffusion coefficient (D) and the lifetime of the activated state (τ).

Fluorescent Translocation Biosensors as Tools in Signal Transduction

Many recent studies have shown that the translocation and subcellular localization of signaling proteins or signaling protein domains can be used as versatile tools to study when and where a particular signaling step is triggered, to dissect activation mechanisms of enzymes, or to investigate chemotactic and other localized signaling responses. In many cases, the localization of multidomain signaling proteins is

dependent on several signaling events and binding interactions. In these cases, **GFP** conjugated minimal domains have been found to be useful tools to monitor a particular signaling step more selectively. For example, GFP conjugated **PH domains** of Akt and GFP conjugated **C1 domains** from **PKC** were found to be more selective and higher affinity probes than the full-length proteins for the visualization of localized changes in plasma membrane 3' Ptdins and diacylglycerol concentration, respectively.

Future Microscopy Signaling Studies

Despite these current limitations, a rapidly growing number of studies are demonstrating that the ability to track signaling processes in space and time opens up new possibilities to dissect the dynamic mechanisms in signal transmission and to gain a spatial and temporal understanding of intracellular signal transduction that would not be possible by using biochemical approaches. With the possibility to now study **CFP** as well as **YFP** (cyan and yellow fluorescent protein) conjugated signaling proteins and domains in the same cells, the subcellular analysis of the distribution of signaling proteins can be made quantitatively. Furthermore, in some cases it will be possible to measure protein-protein binding interactions by **fluorescence resonance energy transfer (FRET)**.

There is now much interest in making the currently low throughput fluorescence imaging approaches better suited to attack the more complex question of how entire signaling networks are coordinated in space and time. In such measurements, selected sets of fluorescence biosensors could be used for measuring intermediate or final signaling steps. Such measurements could then be combined with perturbation strategies using antisense oligonucleotides, pharmacological libraries, or sets of expressed dominant-negative and constitutively active constructs in order to gain insights into the wiring diagram and the feedback time constants of cellular signal transduction networks.