Cellular Signalling 24 (2012) 1899–1908

Contents lists available at SciVerse ScienceDirect

Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

Review

Synthetic biology with surgical precision: Targeted reengineering of signaling proteins

Vsevolod V. Gurevich *, Eugenia V. Gurevich

Department of Pharmacology, Vanderbilt University, Nashville, TN 37232, USA

ARTICLE INFO

Article history:
Received 1 May 2012
Accepted 14 May 2012
Available online 1 June 2012

Keywords:
Cell signaling
Signaling pathways
Arrestin
GPCR
Scaffold protein
Protein engineering
Cell survival
Cell death

ABSTRACT

The complexity of living systems exceeds everything else studied by natural sciences. Sophisticated networks of intimately intertwined signaling pathways coordinate cellular functions. Clear understanding how the integration of multiple inputs produces coherent behavior is one of the major challenges of cell biology. Integration via perfectly timed highly regulated protein–protein interactions and precise targeting of the "output" proteins to particular substrates is emerging as a common theme of signaling regulation. This often involves specialized scaffolding proteins, whose key function is to ensure that correct partners come together in an appropriate place at the right time. Defective or faulty signaling underlies many congenital and acquired human disorders. Several pioneering studies showed that ectopic expression of existing proteins or their elements can restore functions destroyed by mutations or normalize the signaling pushed out of balance by disease and/or current small molecule–based therapy. Several recent studies show that proteins with new functional modalities can be generated by mixing and matching existing domains, or via functional recalibration and fine–tuning of existing proteins by precisely targeted mutations. Using arrestins as an example, we describe how manipulation of individual functions yields signaling–biased proteins. Creative protein redesign generates novel tools valuable for unraveling the intricacies of cell biology. Engineered proteins with specific functional changes also have huge therapeutic potential in disorders associated with inherited or acquired signaling errors.

Contents

1. Introduction

Biological systems are arguably the most complex ones studied by natural sciences. An average animal cell with the diameter of ~10 μm weighing ~1 ng contains >2 billion molecules of hundreds, if not thousands, of different types of proteins, >80 billion molecules of various lipids, plus amino acids, nucleotides, metabolites, ions, etc. Thus, even if we don't count ~20 trillion water molecules, the total number of biomolecules in an average cell exceeds the number of humans that ever lived since our species emerged. According to the laws of thermodynamics, the system of this complexity cannot be static. Indeed, each cell constantly receives and interprets hundreds of various stimuli, adjusting every

* Corresponding author. Tel.: +1 615 322 7070; fax: +1 615 343 6532.
E-mail address: vsevolod.gurevich@vanderbilt.edu (V.V. Gurevich).

0898-6568/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.cellsig.2012.05.012

aspect of its behavior accordingly. Networks of signaling proteins that integrate inputs and coordinate responses govern these changes. Thus, in order to tell the cell what to do, we need to send our message via signaling proteins in a language the cell understands.

Elucidation of the fine molecular mechanisms of cell signaling is one of the greatest challenges of modern biology. The ability to produce expected outcome in a living cell by targeted manipulation of its signaling pathways is the ultimate test of our understanding of the mechanisms governing the cell behavior [1,2]. Such ability may also be the ultimate therapeutic tool enabling us to restore normal behavior in cells where signaling is perturbed by a disease. We should not be embarrassed to acknowledge that we cannot build a living cell with desired functional characteristics from scratch: after all, this achievement took evolution more than a billion years of rigorous experimentation. Nor is it particularly necessary. However, if it were possible to reprogram a malfunctioning cell, thus restoring its normal behavior, it would certainly have a therapeutic value. Small molecule drugs are aiming at achieving just that but as tools they have inherent limitations, primarily because they are not a part of the cellular signaling network and thus are not responsive to regulatory feedbacks. In many cases, drugs offer relief but not a cure. This is particularly obvious in case of neurological and psychiatric diseases, when even in the best case scenario a patient is maintained in a reasonably functional state by drugs, but that means taking the drugs for years without any hope of ever becoming disease-free. Another limitation of drugs is that not all protein functions involved in disease pathogenesis are amenable to regulation by small molecules. Receptors and enzymes are targeted by drugs quite successfully, whereas interfering with protein–protein interactions is much more complicated, particularly when the task is to *enhance* that interaction rather than disrupt it. Regulating by drugs of subcellular distribution, folding, or disposal of proteins involved in the disease process is also not easily accomplished.

An alternative to using small molecules to regulate cellular signaling is to employ signaling proteins as experimental and, ultimately, therapeutic tools. The simplest approach is to regulate the expression level of an endogenous signaling protein by overexpression of a wild type protein or by knockdown with some sort of an RNAi construct. Multiple attempts have been made to employ both these approaches for therapeutic purposes [3–6]. Recent and future advances in viral and non-viral delivery methods will make these techniques a viable clinical option for many diseases. However, manipulating an endogenous signaling protein simply by reducing or increasing its availability would inevitably affect all of its functions, which may not be always desirable or even safe in some cases. Targeted manipulation of specific functions of a multifunctional protein while preserving all other functions intact may be a preferred approach. To this end, mutant proteins with specific functions disabled or enhanced by precisely targeted mutations have to be employed. Furthermore, signaling can be redirected using novel scaffolding proteins assembled from existing domains, or new cellular functions could be created by expression of additional proteins in cells that do not normally express them. The problem is that these approaches require a much more extensive knowledge of structure-functional properties of signaling proteins involved in diseases. First, we need to know which function needs to be manipulated and how; second, we need to be able to construct mutants with desired properties to serve as tools. However, in recent years, a remarkable progress has been made in several such directions with therapeutic potential, largely using strategies previously validated by evolution. Here we overview some of these approaches, with particular focus on reengineering scaffolds to selectively suppress or enhance individual functions, using arrestins as an example of multi-functional organizers of cell signaling.

2. Modulating cell signaling by existing proteins and their elements

Ectopic expression of wild type proteins in sites other than their native location in order to compensate for the loss of function associated with the degeneration of cells that normally bear these proteins is an exciting approach with an enormous therapeutic potential. Recently, its feasibility has been proven experimentally, although admittedly there is still a long road ahead before this technique becomes a viable therapeutic choice. Mutations in dozens of human genes cause various forms of retinal degeneration, with a devastating result of complete blindness [7]. Recent successful gene therapy trials demonstrated that early intervention in case of some loss-of-function mutations can be successful. Three clinical trials attempted to cure Leber congenital amaurosis, which is caused by the deficiency of retinal pigment epithelium 65 (RPE65) [6,8]. This protein performs a key step in the so called visual cycle, the conversion of all-trans-retinal released by light-activated rhodopsin into 11-cis-retinal necessary for rhodopsin regeneration [9,10]. In its absence, rhodopsin cannot be regenerated, which leads to complete loss of rod function. The expression of fully functional RPE65 in retinas that lack this protein dramatically improved photoreceptor function and survival [6,8]. However, the situation was considered hopeless after complete loss of photoreceptor cells. A recent study demonstrated that this is not necessarily the case [11]. Retina consists of multiple types of neurons, light-sensitive photoreceptors being the most prominent. Photoreceptor cells are the most vulnerable, dying off in retinitis pigmentosa and other types of retinal degenerations. The demise of photoreceptors results in blindness, but the other neurons remain in their place, although they undergo extensive rewiring [12]. Restoring vision in cases where photoreceptors are lost is an unmet challenge. Light-activated ion channels were expressed in non-light-sensitive ON bipolar cells in the retina of blind mice that lost photoreceptors due to retinal degeneration [11]. An exciting finding was that this expression conferred sufficient light sensitivity to allow these animals to successfully perform vision-guided behavioral tasks [11]. Although the animals only became sensitive to relatively bright light, this was a vast improvement. This study shows that the expression of an additional protein can generate a new functional modality, such as light sensitivity of bipolar cells, and this “unnatural” signal can be transmitted via existing circuits and successfully used by the brain to guide behavior.

Color blindness is another genetic disorder that was considered incurable. Recent experiments showed that the expression of a third type of cone pigment in photoreceptors of dichromatic adult monkeys successfully provided trichromatic color vision in these animals [13]. It is worth noting that here a particular cone opsin was expressed in the cells that never had it before. It apparently successfully used existing signaling machinery to confer the ability to discern light with specific wavelength to animals that were dichromatic from birth. In both of the above cases, a single additional protein was expressed in existing cells, and the brain was able to correctly interpret this new functional modality and successfully use additional information provided by it.

In many ways, the retina is unique, because it represents a sensory organ and a self-contained highly organized circuitry dedicated to the detection and analysis of the visual signals. This makes it easier for the brain to learn to correctly interpret retinal signals even when they come from the “wrong” cells, because they are partially made sense of at the retinal level due to the built-in properties of the circuit. Such circuit “reprogramming” is likely to be more difficult in other areas of the brain, although it is feasible in cases of well-defined circuit malfunctions. One such example comes from the field of Parkinson's disease (PD). The classic model of PD pathophysiology posits that selective loss of dopaminergic neurons providing dopamine to the striatum leads to reduced activity of the direct and enhanced activity of the indirect output striatal pathway, resulting in a net increase of the inhibitory striatal output to the thalamus and excessive inhibition of the thalamo–cortical network [14,15]. One of the main contributors to such an outcome is believed to be the elevated abnormal activity of the excitatory subthalamic nucleus. Numerous clinical data with deep brain stimulation of the nucleus support the notion that reduction in the activity of the subthalamic nucleus yields improvement in parkinsonian symptoms [16]. Recent report of a successful gene therapy trial [17] based on initial preclinical findings [18] demonstrated that by expressing glutamic acid decarboxylase, a rate-limiting enzyme