Templated Catalysis

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A Molecular Tête-à-Tête Arranged by a Designed Adaptor Protein**

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The tightly controlled and well-balanced cooperation of individual cells is fundamental for the homeostasis of multicellular organisms. Therefore, cells communicate by means of signaling molecules, which induce responses in signaling networks and ultimately lead to altered enzyme activities or changes in gene expression.[1] Protein–protein interactions are crucial for the intracellular propagation of these signals. Protein di- or oligomerization alone or subsequent chemical modification of one protein by the other (through for example, phosphorylation) can thereby be necessary to cause a specific signaling outcome.[1,2] The detailed comprehension of these processes is crucial to understanding the function and malfunction of extensively intertwined signaling pathways, the latter of which is often a cause of severe human diseases.[1,3]

Approaches to deciphering signaling pathways and identifying the involved proteins include probing the system with inhibitors and genetic approaches such as RNA interference,[4] performing co-immunoprecipitations, and applying diverse imaging techniques.[5] Another method is using chemical inducers of dimerization (CIDs), which are cell-permeable molecules that initiate specifically the homo- or heterodimerization of target proteins.[6] By genetically fusing the binding domains, such as FK506 binding protein (FKBP) and the FKBP-rapamycin binding domain (FRB), of a chemical dimerizer, for example rapamycin, to a pair of proteins, virtually any combination of target proteins can be brought close together inside the cell.[6] This principle has been used elegantly to assess the role of distinct protein-binding events of specific proteins in cell signaling pathways.[6] Finally, the field of synthetic biology has already generated a bottom-up approach for deciphering signaling pathways in which cellular circuits are rewired using genetic approaches, for example the fusion of scaffold proteins to bring network components together that would usually not interact.[7]

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In their recent publication, Schepartz and Hobert devised a novel way to induce proximity between proteins.[8] By joining two miniature proteins in one molecule, a synthetic adaptor protein was created that led to the formation of a ternary complex between the adaptor, a kinase, and a noninherent substrate, and resulted in the enzymatic modification of one binding partner by the other (Figure 1 A).[8] In analogy to naturally occurring adaptor proteins these molecules were termed synthetic adaptor proteins.[8]

Miniature proteins are compact polypeptides consisting of less than 50 amino acids with well-defined, typically helical, secondary structure elements.[8,9] They are genetically encodable and evolvable, as well as—owing to their small size—synthetically accessible.[8] For their development single domains of larger proteins exhibiting the desired biochemical properties are used as templates.[9] Further optimization of sequence and structure has been shown to provide molecules that have superior characteristics compared to the parent proteins in terms of potency and off-target effects.[8,9]

In earlier work the Schepartz group had reported the development of miniature proteins that bind the Src family kinase Hck (YY2)[9a] and the physiological negative regulator of p53 activity hDM2 (3.3) selectively and with high affinity in the low micro- or nanomolar range.[9b] YY2 (4.4 kDa) was developed on the basis of a peptide binding the SH3 domain of c-Src, which was grafted into the polyproline II (PPII) helix of the small, but structurally very stable avian pancreatic polypeptide (aPP) to interact with the SH3 domain of Src family kinases, and it was shown to bind and activate Hck in vitro.[9a] Miniature protein 3.3 (4.8 kDa) was engineered by the insertion of residues into the α-helical segment of aPP, which are critical for the binding of the activation segment of p53 (p53AD) to hDM2. Subsequent sequence optimization of residues not involved in binding or helix formation by phage display led to the identification of 3.3 as the strongest binder to hDM2 in the library.[9b] It is capable of inhibiting the formation of the p53AD–hDM2 complex in vitro and, thus, holds promise to act as a genetically encodable activator of p53 in vivo.[9b,10]

Connecting the two miniature proteins YY2 and 3.3 in one molecule through an optimized linker generated adaptor proteins that retained the ability to bind Hck and hDM2 and induced the formation of a ternary complex in vitro, as shown in fluorescence polarization and pulldown experiments.[8]

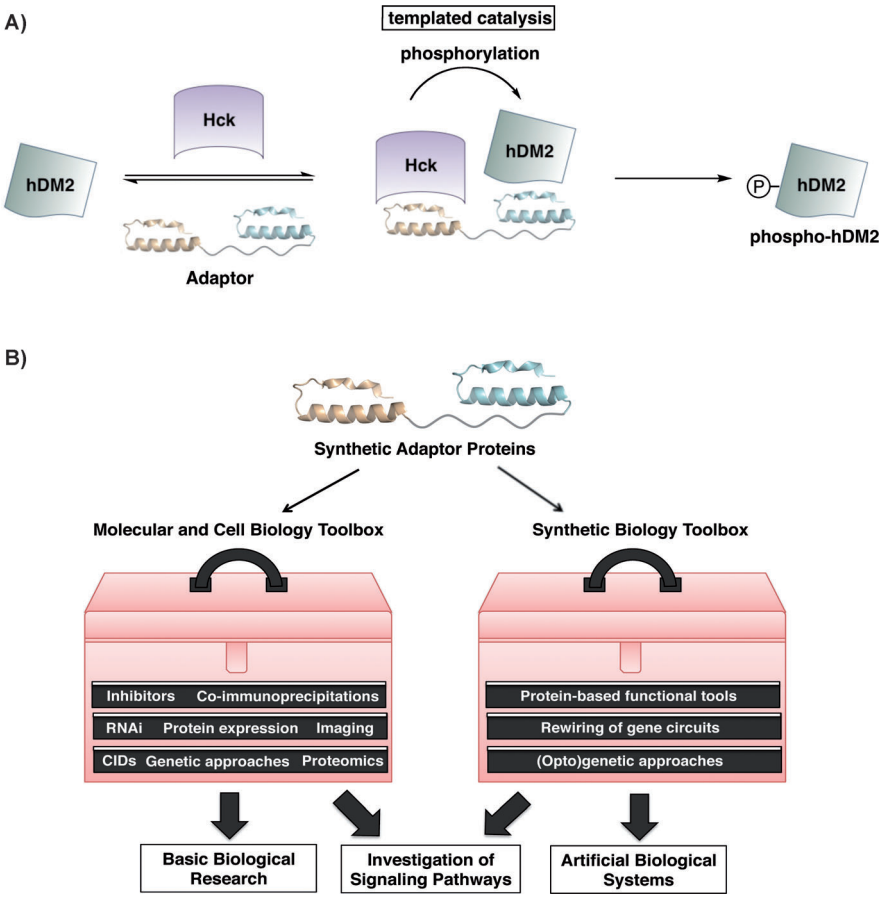

Figure 1. Principle and potential applications of adaptor proteins. A) Formation of a ternary complex and subsequent proximity-induced phosphorylation. B) Synthetic adaptor proteins will expand the toolbox used to decipher signaling pathways and explore synthetic biology.

Moreover, addition of the adaptors to a mixture of Hck and hDM2 led to an increase in the phosphorylation of hDM2 up to twofold in in vitro experiments.[8] Interestingly, hDM2 is a physiological substrate of the c-Abl kinase and represents a poor Hck substrate under normal circumstances. However, bringing the two proteins close together enhanced substrate turnover.[8] This principle of proximity-induced catalysis is well known and has been used for multiple applications such as DNA-templated and target-guided synthesis.[11] Using LC/MS/MS and Western blot analysis, Y405 was identified as the major phosphorylation site on hDM2 by Hck.[8]

Currently the Schepartz group is investigating whether the adaptor protein can induce the phosphorylation of hDM2 in vivo and thereby release its inhibitory activity on p53, resulting in the transcription of p53-dependent genes. This would represent the proof-of-principle for the in vivo applicability of this new concept, which can potentially be used for many more protein–protein interactions given the widespread use of modular domains in signaling proteins.[1]

A comparison of this new method to the established CID approach shows that one advantage of the former is that the proteins of interest do not need to be genetically modified or overexpressed. This is the case in the CID approach, where genetic fusions to the dimerizer binding domains are necessary.[6] On the other hand, the CID system is currently more generally applicable and does not rely on the presence of targetable binding domains and the development of a specific adaptor protein to study the desired interaction.[6]

The possibility of genetically encoding the adaptor protein directly in the respective model system avoids potential problems caused by cell penetration and stability.[8] However, especially in experiments related to signal transduction, a faster inducible and reversible effect might be desired. The strategy of peptide stapling, which relies on the stabilization of α-helical domains by insertion of an all-hydrocarbon cross-linker, has been used successfully to develop a helical peptide that also binds hDM2 and reactivates the transcription of p53-dependent genes.[12] Stapled peptides can enter cells through an active transport mechanism and,[12] therefore, the conjoining of two stapled peptides to create a stapled adaptor protein appears to be a potential way to create cell-permeable adaptor proteins, enabling more rapid cellular responses and complementing the usage of genetically encoded adaptor proteins.

Finally, the combination of synthetic adaptor proteins with the CID technology could lead to the creation of an artificial phosphorylation–dephosphorylation system in cells. The adaptor protein, connecting a kinase and the target protein of interest, would be expressed fused to, for example, the FRB domain. This would result in the rewired phosphor-

ylation of the desired target. At the same time, a suitable phosphatase would be expressed fused to the FKBP domain. Upon addition of rapamycin, the phosphatase would be brought in close proximity to the phosphorylated target and could counteract the kinase, balancing the phosphorylation level.

The concept of synthetic adaptor proteins as inducers of protein–protein interactions and modifications holds great promise to become a valuable addition to the toolbox of methods in cell and synthetic biology (Figure 1 B), if their in vivo applicability can be confirmed. Redirecting enzyme activities to latent substrates is another exciting example of templated catalysis. This tool can help dissect interconnections within and between signaling pathways, and can in this way contribute to a deeper understanding of the interactions in the complex signaling networks regulating all the vital processes in living organisms.

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