doi:10.1246/cl.2012.658

Published on the web June 23, 2012

Highlight Review

Chemical Biology of Natural Products on the Basis of Identification of Target Proteins

Minoru Ueda

(Received March 14, 2012; CL-120224)

Abstract

Recent progress in chemical biology research has accelerated the target protein identification of biologically active small molecules, including naturally occurring ligands. It is widely recognized that a biologically active natural product functions as a "key" that fits a specific protein "lock." However, recent studies have revealed multiligandable nature of natural products that works as "multiple keys" (or a "master key") fitting multiple "locks" in a biological event. Biological experiments using natural products as ligands have often provided puzzling results, which can likely be attributed to their multiligandability. Thus, a novel research direction dealing with multiliganbale natural product ligand based on their target identification is strongly desired. This review focuses on the current status of natural products chemistry based on their target identification, and suggest the future direction, named chemical biological chemistry (Chem-Bio-Chem).

♦ Introduction

Natural products chemistry has supplied a myriad of compounds with complex structures as well as unique biological activities. Structural determinations and chemical syntheses of complex natural products (Figure 1) led to new concepts in organic chemistry; studies on hinokitiol,[1] steroids,[2] vitamin B_{12},[3] ginkgolide,[4] palytoxin,[5] ciguatoxin,[6] and maitotoxin,[7] inspired non-benzenoid aromatics,[1] conformational analysis,[2] Woodward–Hoffmann rules,[8] and the nuclear Overhauser effect (NOE) in structure determination.[4] Such chemical research on natural products had been highly successful until the 1980s, and contributed greatly to the development of organic chemistry. Unfortunately, such research became less productive in the 1990s. For example, Seebach became suspicious of the "primary motivation" of natural product synthesis as early as 1990.[9]

It has become significantly more difficult to find examples of novel natural products or their total syntheses that have provided new concepts in chemistry, as if such conventional structure-based research has been exhausted. This review introduces a new strategy of natural products chemistry on the basis of molecular targets involved in the mode-of-action of naturally occurring ligands. This is an important paradigm shift "from structure to biological activity" in natural products chemistry. In this review, natural products are renamed as naturally occurring ligands (NOLs) to underline their biological importance.

♦ "Multiple Keys:" A Multiligandable Nature of Natural Products

It is widely recognized that target exploration of NOLs is an

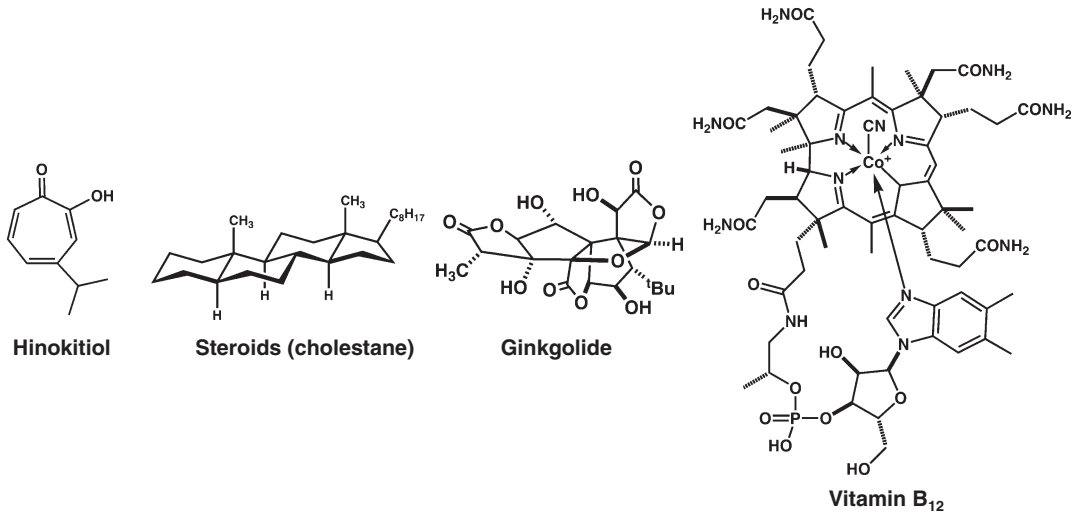

Figure 1. Natural products inspired new concepts in chemical research.

Prof. Minoru Ueda
Department of Chemistry, Tohoku University, 6-3 Aramaki-Aza Aoba, Aoba-ku, Sendai, Miyagi 980-8578
E-mail: ueda@m.tohoku.ac.jp

immense undertaking, although many successful examples of target identification of small molecules have been reported.[10,11] This difficulty can be partly attributed to the complexity underlying the nature of NOLs. The "lock-and-key" model is widely recognized as a fundamental concept that explains the biological activity of NOLs: a molecule interacts with a specific target in vivo and turns on its unique biological activity. However, it has gradually been recognized that such a simple concept cannot fully explain the biological activity of all NOLs. Recent studies suggest that we are observing the sum of plural biological activities of NOLs,[12,13] and it is more suitable to use an analogy of "multiple keys" (or a "master key") that can fit several "locks" to explain the multiligandable nature of NOLs (Figure 2).

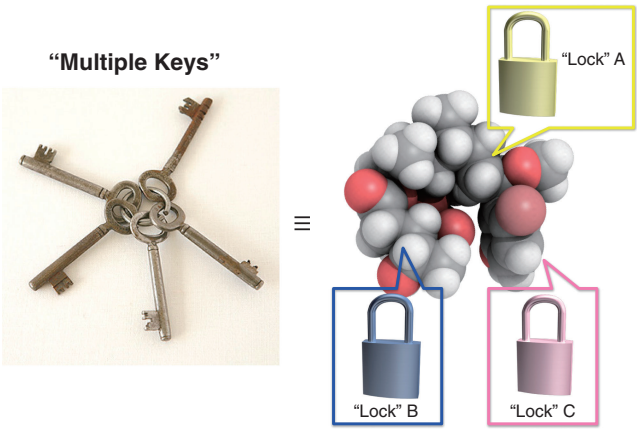

Figure 2. "Multiple keys:" Multiligandable nature of natural products (naturally occurring ligand: NOL).

♦ *Target ID Enables a Rational Molecular Design of Truncated NOL*

One of the scientific merits in target identification (target ID) of NOLs lies in the potential contribution to a new strategy for molecular design. Recently, many attempts have been made to downsize NOLs with retention of their unique bioactivities.[14] Trimming the fat by removing some moieties from complex molecules as well as reducing excess functionalities can generate simple, truncated NOLs. Such simplification, an extraction of the essential structure for specific activity, will decrease the number of target proteins and will serve to simplify the biological activity of the NOL. The resulting simplified NOL often retains its original bioactivity to some extent and can be considered to be a drug candidate or a useful clear-cut biochemical reagent without exhibiting side effects. The most successful achievement of this strategy can be found in the case of eribulin (Halaven),[15–17] which is a truncated derivative of halicondrin B,[18] a potent antitumor marine natural product (Figure 3). Wender's approach using bryostatin is another successful example of a truncated NOL.[19–22] Thus, a promising future is expected for this approach. However, the most serious issue here is that there is no reasonable way to predict the essential structure of a complex NOL. A tremendous amount of effort is necessary for the "extraction" of an essential structure from a NOL. For example, eribulin was developed after as many as 200 derivatives of halichondrin B were synthesized and evaluated.[23] Thus, the establishment of reliable guidelines to elucidate the core structure of a NOL is highly desired.

The target ID of NOLs can provide such guidelines. The dissection of "various keys" (or a "master key") based on the 3D structure of a ligand/target complex will provide precise information on the binding site of the ligand. This information will lead to the rational design of the essential structure of a

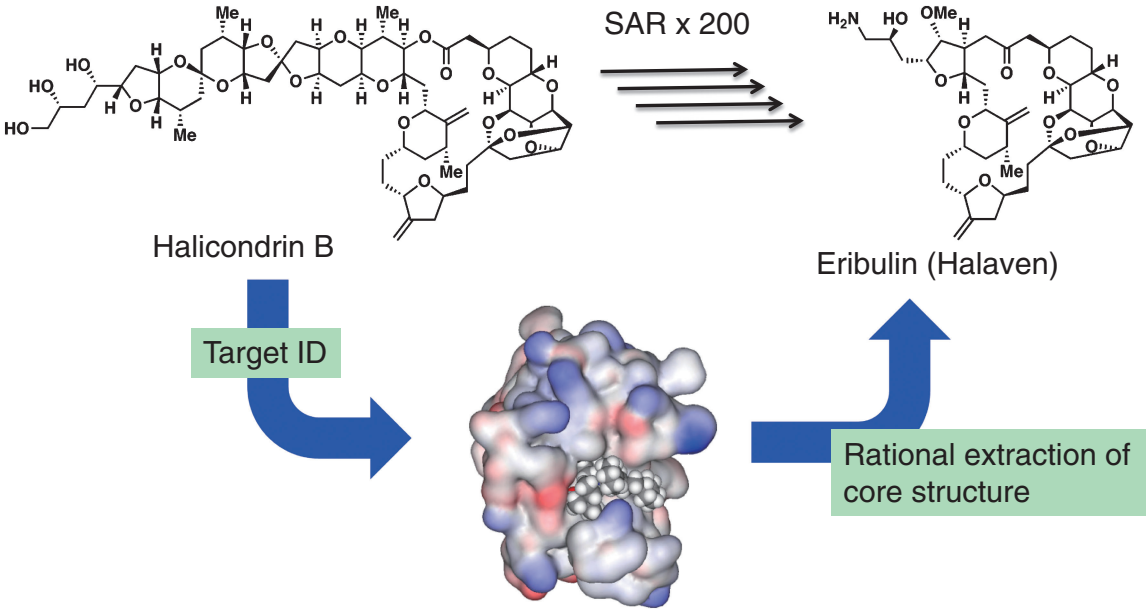

Figure 3. An example of development of a "truncated" naturally occurring ligand (NOL) by conventional SAR approach, and a new approach for rational design based on target protein identification.

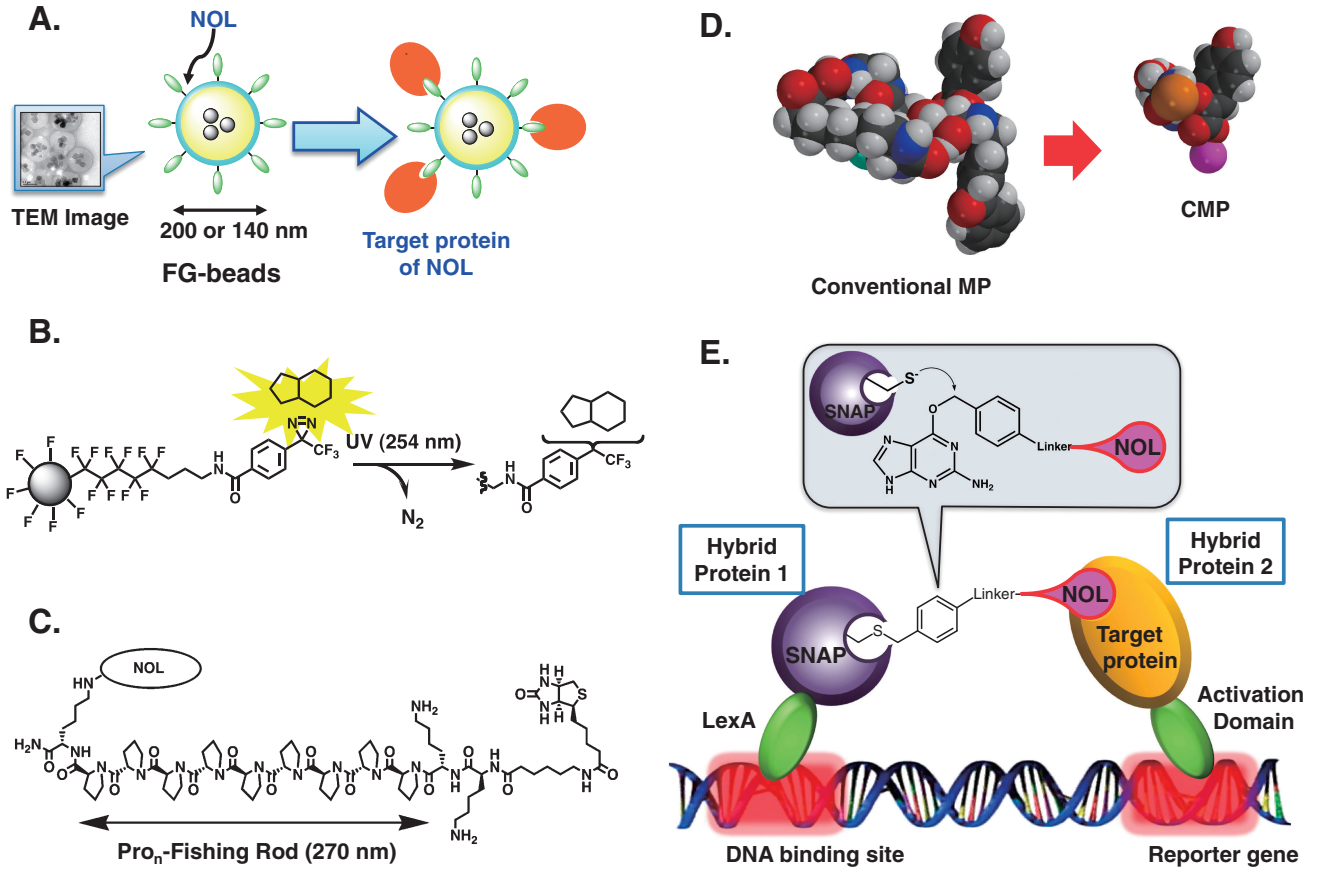

Figure 4. Methods for target ID of small molecules: A) FG-beads technology, B) photoaffinity beads technology, C) fishing-rod strategy, D) compact molecular probe (CMP) strategy, and E) Y3H combined with SNAP-tag technology.

NOL corresponding to a single "key" against a specific "lock." The 3D mapping of binding sites in whole ligand structures can be obtained precisely by X-ray crystallography and approximately by NMR saturation transfer experiments.[24,25] However, the most serious issue preventing such an approach is the difficulty in the target ID of NOLs.

♦ *Methods for Target ID*

Identification of each "lock" corresponding to a desired bioactivity is essential for the above purpose. Recent progress in chemical biology brings us some promising methods applicable for target ID of NOLs, such as FG-beads technology,[26] photoaffinity beads technology,[27] a fishing-rod strategy,[28] a "compact" molecular probe (CMP) approach,[29] the yeast 3-hybrid (Y3H) system,[30] and a database-dependent (DBD) strategy[31,32] (Figure 4). Historical background of these techniques can be found in excellent reviews.[10,11] However, the potential of these methods in the target ID of NOLs has not been fully explored.

FG-bead and photoaffinity bead technologies are types of affinity chromatography with unique modifications. FG-bead technology[26] developed by Handa and co-workers (Figure 4A) is widely applied for the target ID of small molecular drugs. The small size of the magnetic beads (140–200 nm diam.) enables a greater quantity of effective ligands to be placed on the beads, providing strong improvement in the efficiency of affinity chromatography.[26] Additionally, the small nonspecific binding nature of FG beads yields reliable results in affinity chromatography. The most successful achievement by FG-bead technology is the target ID involved in a side effect of thalidomide, a tranquilizer and painkiller. This is a beautiful example in which the "multiple keys" nature of thalidomide was revealed by the identification of cereblon, a component of E3 ubiquitin ligase.[33]

Photoaffinity beads technology[27] (Figure 4B) developed by Osada and Kanoh is widely applied for the target ID of NOLs.[34] The most important advantage of this technique is that it requires no prior structure–activity-relationship (SAR) studies. In order to obtain successful results using standard affinity beads, the NOL should be immobilized on the beads without loss of its affinity with the target protein. Thus, SAR studies are indispensable to determine which functionality is irrelevant to the bioactivity of the NOL, and can therefore be used for connecting the NOL to the beads. SAR studies are a time-consuming, but essential step for the preparation of affinity beads. In contrast, in photoaffinity beads technology, random immobilization of the NOL was achieved by using beads equipped with a linker in which trifluoromethyldiazirine (TFMD) is connected. The random immobilization provides an array of beads photocrosslinked to random sites of the NOL, which encompasses theoretically all possibilities of binding to the corresponding target proteins.

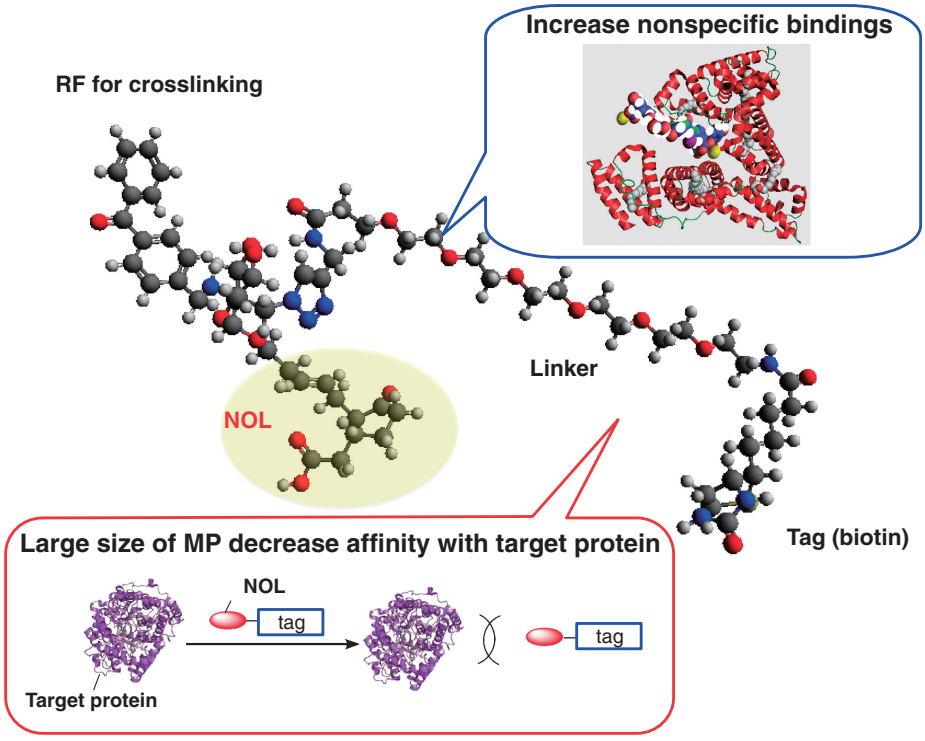

Figure 5. Typical structure of a molecular probe (MP) equipped with a reactive functionality (RF) as well as a tag (biotin) with linker.

The fishing-rod strategy (Figure 4C) and compact molecular probe (CMP) approach (Figure 4D) are molecular probe (MP) methods. An MP is a modified NOL equipped with a reactive functionality (RF) for crosslinking as well as tags for detection and separation (Figure 5).[35] Tags, such as biotin or epitope peptides, are usually connected to the NOL via a linker moiety, such as an oligopeptide or poly(ethylene glycol) (PEG).[36] MP technology is a conventional method for target ID of small molecules;[37] however, it sometimes gives enigmatic results due to the presence of many nonspecific bindings. An important problem observed in target ID using a molecular probe lies in the existence of the linker and tag moieties, which may decrease the affinity with the target as well as cause many nonspecific bindings (Figure 5). Thus, an appropriate control experiment should accompany the target ID. Competitive inhibition studies are indispensable for the confirmation of the target ID.[38] The use of a negative probe for which no bioactivity is observed would be the most reliable. As shown in Figure 6, an enantiomer (jasmonate glucoside (JAG)/*ent*-jasmonate glucoside (*ent*-JAG))[39] or epimer (aurilide/6-*epi*-aurilide)[40] of the NOL provides successful results in their target IDs because these species have almost the same physical properties as the positive probe and can be employed as a perfect control. However, more reliable technology without nonspecific binding is highly desired to establish a useful method for robust target ID. The fishing-rod strategy and CMP approach have been devised to reduce the troublesome nature of the MP approach.

The fishing-rod strategy[28] developed by Uesugi and co-workers (Figure 4C) employs a unique rigid polyproline linker. Uesugi focused on the 3D structure of the MP to improve its troublesome nature. A photoaffinity probe with a rigid polyproline linker moiety can cast the tag moiety away from the ligand moiety and improve the affinity with the target. It is also advantageous in improving the signal (target)-to-noise (nonspecific bindings) ratio in the target ID experiment. The fishing-rod strategy was successfully applied for the identification of the novel target of indomethacin, GLO-1.[28]

The CMP approach[29] developed by Ueda and co-workers (Figure 4D) is a state-of-the-art technology for the target ID of NOLs. This concept is based on the idea that the NOL itself has the best affinity with its target and any modification of a functional probe will decrease its original affinity. The combination of this small-sized affinity probe and copper-catalyzed azide–alkyne cycloaddition (CuAAC)-based[41,42] stepwise introduction of the FLAG-epitope tag is expected to give a good signal-to-noise (nonspecific bindings) ratio in target ID experiments. The CMP approach provided a perfect result in which only the specific target was tagged without any nonspecific binding.[29] These successful results were obtained in the target IDs of isolespedezic acid and jasmonate glucoside. A similar concept employing a unique double-click strategy was also developed by Hosoya.[43,44]

Moreover, it should be mentioned that significant efforts have been made for the improvement of reactive functionalities (RFs) that are used for forming crosslinks between a target protein and a molecular probe. Trifluoromethyldiazirine (TFMD)[45,46] and benzophenone[47,48] have been widely used as photoaffinity RFs, however, their crosslinking yields are estimated to be as low as a few to several percent. Thus, conventional electrophilic RFs, such as iodoacetyl, epoxides, or α,β-unsaturated ketones, which covalently crosslink to a target protein, have been reevaluated because reaction yields using these species are often much higher than those using photoaffinity RFs.[49] Recently, the advantage of tosyl chemistry[50] was

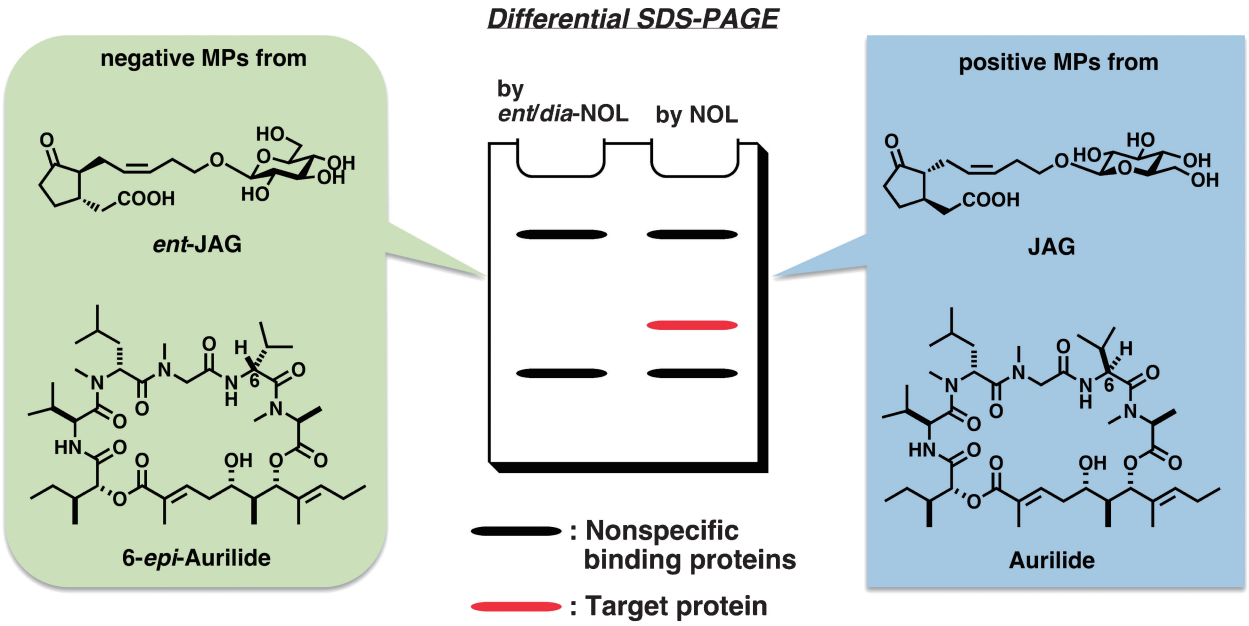

Figure 6. Differential SDS-PAGE analysis: an enantiomer or diastereomer (epimer) is used as a negative control. The red-colored target protein can be easily discriminated from black-colored nonspecific binding proteins.

demonstrated by Hamachi; however, its application for NOL has unexamined.

A yeast 3-hybrid system (Y3H)[30a] developed by Liu and co-workers is a recently developed method for the target ID of small molecules, which is a modified version of conventional Y2H technology. More recently, Johnsson[30b] combined this Y3H with SNAP-tag technology to improve its usefulness (Figure 4E). Y3H systems rely on LexA or Gal4 as DNA binding domains (DBD) to which SNAP-tags are added. Small molecules as "bait" can be easily introduced to this SNAP–DBD fusion protein by using an O^6-benzylguanine (BG) derivative. The interaction with the "fish," a transcriptional activation domain fused to a protein from a cDNA target, can be explored with gene expression. Binding proteins, including the genuine target for the bait, will give an expression of the reporter gene. Unfortunately, the application of Y3H seems to be restricted to identification of cytosolic targets.[51]

The database-dependent (DBD) strategy is a totally different method for target ID of NOLs. It is a method utilizing various DBs such as DNA microarrays, proteomes, cancer cell line sensitivities,[31] localizomes,[32a,32b] or zebra fish behavioral phenotype screens on cell responses against small molecules.[52] The localizome approach, in which colocalization of the NOL and target in a living cell is examined, requires fluorescence-labeled (FL) NOLs. However, the large, lipophilic, or charged nature of the fluorophore sometimes affects the localization of FL-NOL. Sodeoka developed Raman imaging of an alkyne-tagged ligand to examine the localization of the ligand.[53] Small alkyne-tags are expected to have little effect on the localization of the original ligand, thus this approach will be highly useful in the localizome approach for target exploration. The forward genetics approach using mutants deprived of responses for NOLs would be included in this category.[54,55] This DBD approach is considered to be one of the most promising. However, serious issues of this approach lie in its strong dependence on generation of a mutant strain as well as a highly organized DB of an entire genome, thus its effectiveness is severely restricted. Successful results using this approach can be found in cases of plant hormones with various mutants of *Arabidopis thaliana*.[55]

In all these methods, reduction of nonspecific bindings or false-positives and retention of binding affinity between the NOL and the corresponding target are common issues in target ID of NOLs. In particular, target ID may become extraordinarily difficult in cases where NOLs have weak or moderate affinity with the target or the target is a protein present at trace levels. Improvements in both the weak points will give us a universal method for target ID of NOLs.

Additionally, it should be remembered that the most serious issues of target ID lie in the lack of a universal strategy for target validation which is the most difficult issue to be conquered. Target validation is a functional characterization of target protein obtained by affinity-based biochemical techniques. This is a crucial process for the identification of receptors because affinity-based biochemical purification of putative target often gives "nonspecific binding proteins" which possess physico-chemical affinities to NOL without any involvement in desired biological activity. One of the most effective strategies for the target validation lies in information concerning the physiological importance of the identified target, such as observation of the phenotype in a knockout or knockdown mutant, the time-course change of target expression, and localization in the whole body. Reverse genomic techniques, such as knockout or knockdown of the desired gene, are the most powerful tools for this purpose; however, these techniques can be applied only in restricted organisms. Localization of the putative target protein on the organ of physiological importance would be one of the most important clues for target ID that can be applicable for nonmodel organisms. For example, information on the specific gene expression in the male antenna of *Bombix mori* provided an

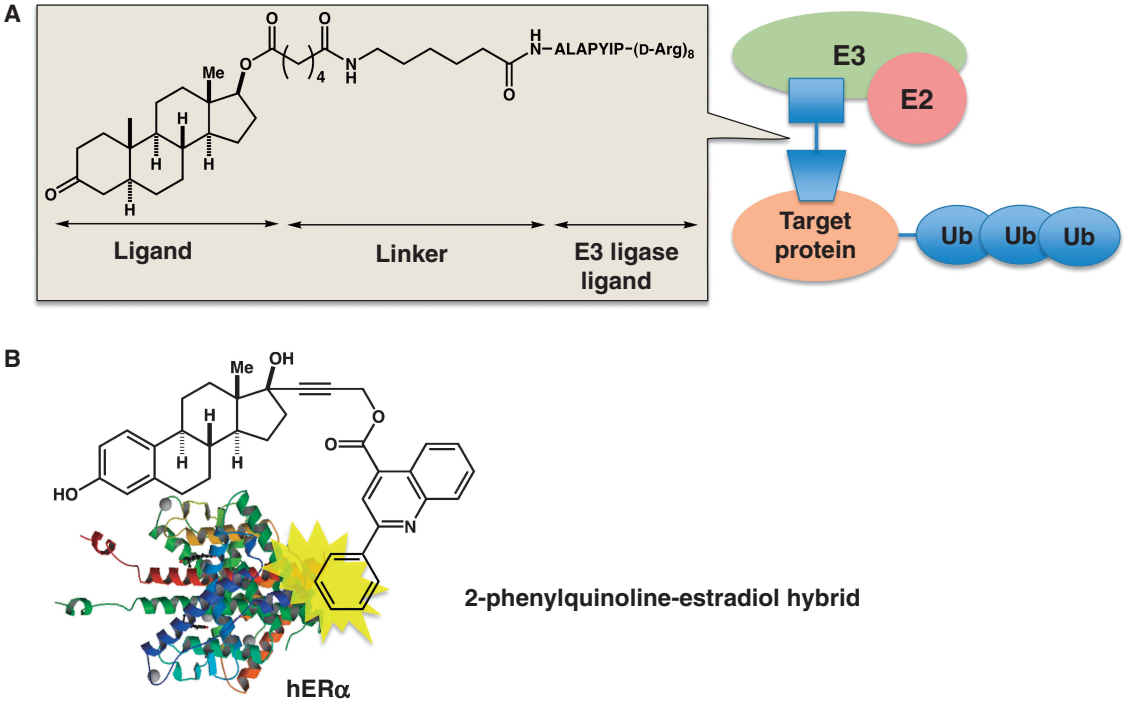

Figure 7. A) PROTAC and B) photoinduced protein degradation strategies; Ub means ubiquitin.

important clue for the target ID of bombykol, a sex-pheromone secreted from the female.[56,57] Bombykol induces the heterodimer formation between bombykol receptor (*Bombix mori* Orfactory Receptor-1: BmOR-1) and BmOrco (*Bombix mori* Orfactory Coreceptor-1) which functions as a ligand-gated ion channel and triggers the generation of action potential.[57] More recently, a gustatory receptor of swallowtail butterfly was identified on the basis of the female tarsi specific expressed genes with a plural transmembrane region.[58]

Recently, some efforts toward the chemical knockdown of desired target proteins have been developed (Figure 7). Proteolysis targeting chimeras (PROTAC)[59,60] developed by Crews (Figure 7A) or protein knockdown[61] developed by Hashimoto is a well-devised method that utilizes an in vivo mechanism for protein degradation. The PROTAC probe forms a bridge between the target molecule and E3 ligase. Ubiquitination (Ub) of the target leads to protein degradation in the proteasome. Toshima[62,63] also developed a photodegradation system for protein knockdown in which a light-activated small molecule, such as phenylquinoline,[62] was connected to a ligand that decomposed the target protein (Figure 7B). Application of these chemical techniques might compensate for the deficiencies of reverse genomic techniques. However, the efficiency of these state-of-the-art techniques is not as high as suppressing the phenotype of the whole body or living cell response.

♦ *Target ID Leading to Discovery of Novel Mechanism on the Regulation of Bioactivity*

Sometimes, target ID of NOL will lead to a discovery of a novel mechanism for the regulation of its bioactivity. A single NOL can bind to plural receptors to induce different responses. Considering its role in vivo, an endogenous NOL, such as hormones, should function depending on the situation by selecting a single "key" from the "multiple keys (or master key)." An unknown in vivo mechanism would operate in such selection of desired bioactivity depending on the situation. For example, in vivo modification of NOLs, such as phosphorylation, oxidation, alkylation, or glycosylation, can drastically change the bioactivity of the NOL as well as corresponding target protein. Additionally, secretion of NOL by a specific efflux transporter shifts the working place of the NOL from the cytosol to the cell surface, and this shift of localization can lead to a drastic change in the target protein.

Recently, we discovered such a novel in vivo mechanism for the regulation of multiple-key nature of endogenous NOL. Glucosides are not considered to be important metabolites in living systems. It has been widely recognized that glucosides are synthesized as storage or waste forms of NOLs. However, our recent results suggest that a glucoside functions as another activated form of a NOL. We found that jasmonate glucoside, which is a glucoside of jasmonate (an oxylipin-type phytohormone), functions in a totally different mode of action as does the original ligand (Figure 8).[64] Jasmonate induces formation of a protein complex between COI1 and JAZ in cytosol to trigger gene expressions concerning various bioactivities,[65–67] whereas jasmonate glucoside cannot bind to the COI1-JAZ receptor complex, but binds to a target membrane protein MTJG[39,64] to induce a shrinking of the cell that leads to a folding of leaves in the *Samanea saman* plant. We named this novel mechanism for the regulation of NOL bioactivity "*glycosylation switching*," because drastic transformation of bioactivity from the original ligand into its glucoside form behaves much like the flipping of a switch. Thus, our finding strongly suggests that glycosylation of a NOL can completely switch the biological activity of the

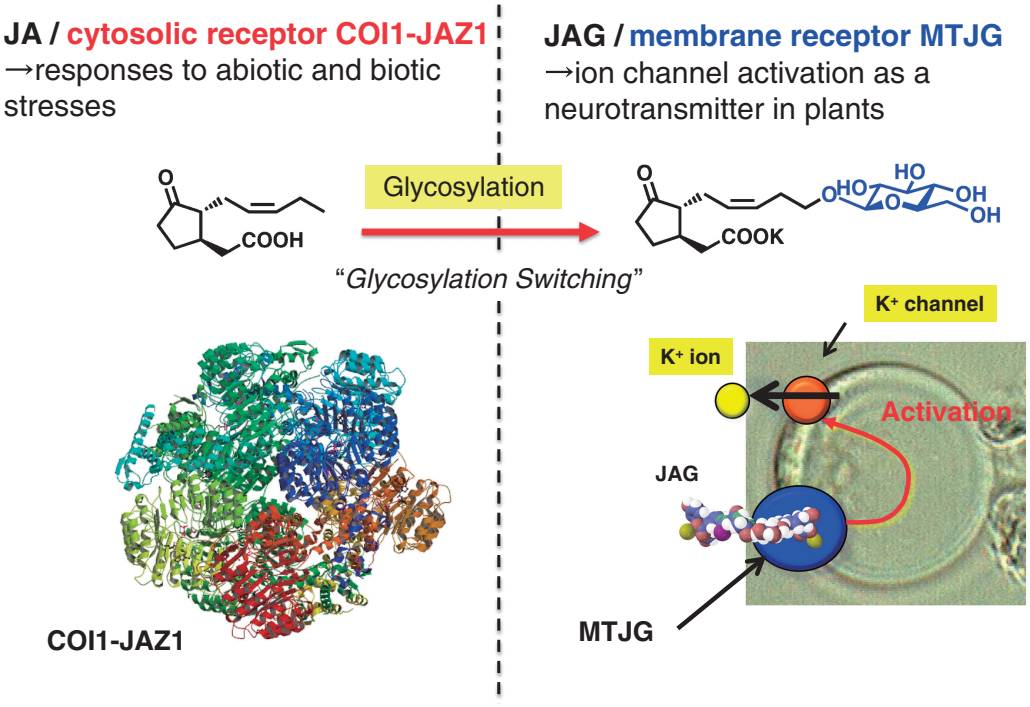

Figure 8. "*Glysosylation switching*" from jasmonate (JA) to jasmonate glucoside (JAG).

NOL. This is an unexpected and surprising role of glycosylation in the regulation of NOL bioactivity.

Target ID of NOL can bring about such unexpected findings on the regulation mechanism of bioactivity. As shown here, chemical biology research (target ID of NOL) can provide clues for new chemical mechanisms to control bioactivity.

♦ *Natural Products Chemistry toward Chemical Biological Chemistry*

The importance of chemical biological chemistry lies beyond the results of chemical biology research that was discussed in 2007 by Isobe.[68] He insisted that one should place priority not only on the target ID, but on new concepts obtained from the result. This suggestion prompted us to explore a new field, chemical biology of natural products, in which we can study new concepts for the regulation of bioactivity dealing with the multitarget nature of natural products.

This new sequence of natural products chemistry based on molecular targets heralds a paradigm shift of strategy "from structure to biological activity." "*Glycosylation switching*" is one gift from such a strategy, which suggests a possible novel mechanism for the regulation of bioactivity operating in vivo. Additionally, the multitarget nature of a natural product provides the possibility of a drastic change in bioactivity by structural modification. Irie and co-workers succeeded in the transformation of bioactivity of aplysiatoxin from tumor-promotion to tumor-suppression by the removal of some functionalities (Figure 9).[69] Aplysiatoxin is known to be a powerful activator of protein kinase C resulting in tumor promotion;[70] however, aplog-1, a truncated analog of aplysiatoxin, binds to an unknown target protein to induce proliferation arrest of tumor cells.

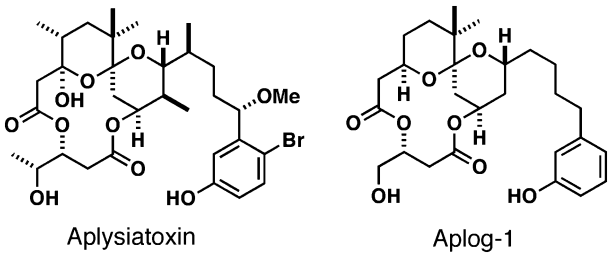

Figure 9. Aplysiatoxin and its "truncated" analog, aplog-1.

Our strategy will provide a new concept in the chemical regulation of bioactivity of NOLs and new ideas on the rational molecular design of modified NOLs for a specific activity. Future scenarios in natural products chemistry will be changed by the introduction of new chemistry from the achievement of chemical biology, now considered to be chemical-biological-chemistry.

References

1 T. Nozoe, S. Katsura, *Yakugaku Zasshi* **1944**, *64*, 181.
2 D. H. R. Barton, *Cell. Mol. Life Sci.* **1950**, *6*, 316.
3 R. B. Woodward, *Pure Appl. Chem.* **1973**, *33*, 145.
4 M. C. Woods, I. Miura, Y. Nakadaira, A. Terahara, M. Maruyama, K. Nakanishi, *Tetrahedron Lett.* **1967**, *8*, 321.
5 D. Uemura, K. Ueda, Y. Hirata, *Tetrahedron Lett.* **1981**, *22*, 2781.
6 M. Murata, A. M. Legrand, Y. Ishibashi, M. Fukui, T. Yasumoto, *J. Am. Chem. Soc.* **1990**, *112*, 4380.
7 M. Murata, H. Naoki, S. Matsunaga, M. Satake, T. Yasumoto, *J. Am. Chem. Soc.* **1994**, *116*, 7098.
8 R. Hoffmann, R. B. Woodward, *Acc. Chem. Res.* **1968**, *1*, 17.

9 D. Seebach, *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1320.
10 a) T. Böttcher, M. Pitscheider, S. A. Sieber, *Angew. Chem., Int. Ed.* **2010**, *49*, 2680. b) M. J. Evans, B. F. Cravatt, *Chem. Rev.* **2006**, *106*, 3279. c) N. Jessani, B. F. Cravatt, *Curr. Opin. Chem. Biol.* **2004**, *8*, 54.
11 a) S.-i. Sato, A. Murata, T. Shirakawa, M. Uesugi, *Chem. Biol.* **2010**, *17*, 616. b) B. J. Leslie, P. J. Hergenrother, *Chem. Soc. Rev.* **2008**, *37*, 1347.
12 S. A. Rizvi, D. S. Courson, V. A. Keller, R. S. Rock, S. A. Kozmin, *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 4088.
13 V. Dančík, K. P. Seiler, D. W. Young, S. L. Schreiber, P. A. Clemons, *J. Am. Chem. Soc.* **2010**, *132*, 9259.
14 a) J.-Y. Wach, K. Gademann, *Synlett* **2012**, 163. b) G. T. Carter, *Nat. Prod. Rep.* **2011**, *28*, 1783.
15 W. Zheng, B. M. Seletsky, M. H. Palme, P. J. Lydon, L. A. Singer, C. E. Chase, C. A. Lemelin, Y. Shen, H. Davis, L. Tremblay, M. J. Towle, K. A. Salvato, B. F. Wels, K. K. Aalfs, Y. Kishi, B. A. Littlefield, M. J. Yu, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5551.
16 M. J. Towle, K. A. Salvato, J. Budrow, B. F. Wels, G. Kuznetsov, K. K. Aalfs, S. Welsh, W. Zheng, B. M. Seletsky, M. H. Palme, G. J. Habgood, L. A. Singer, L. V. DiPietro, Y. Wang, J. J. Chen, D. A. Quincy, A. Davis, K. Yoshimatsu, Y. Kishi, M. J. Yu, B. A. Littlefield, *Cancer Res.* **2001**, *61*, 1013.
17 K. L. Jackson, J. A. Henderson, A. J. Phillips, *Chem. Rev.* **2009**, *109*, 3044.
18 Y. Hirata, D. Uemura, *Pure Appl. Chem.* **1986**, *58*, 701.
19 P. A. Wender, K. F. Koehler, N. A. Sharkey, M. L. Dell'Aquila, P. M. Blumberg, *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4214.
20 P. A. Wender, C. M. Cribbs, K. F. Koehler, N. A. Sharkey, C. L. Herald, Y. Kamano, G. R. Pettit, P. M. Blumberg, *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7197.
21 P. A. Wender, J. DeBrabander, P. G. Harran, J.-M. Jimenez, M. F. T. Koehler, B. Lippa, C.-M. Park, C. Siedenbiedel, G. R. Pettit, *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6624.
22 P. A. Wender, J. L. Baryza, S. E. Brenner, B. A. DeChristopher, B. A. Loy, A. J. Schrier, V. A. Verma, *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 6721.
23 D. J. Newman, *J. Med. Chem.* **2008**, *51*, 2589.
24 M. Mayer, B. Meyer, *J. Am. Chem. Soc.* **2001**, *123*, 6108.
25 Y. Fukunishi, Y. Mizukoshi, K. Takeuchi, I. Shimada, H. Takahashi, H. Nakamura, *J. Mol. Graphics Modell.* **2011**, *31*, 20.
26 a) K. Nishio, Y. Masaike, M. Ikeda, H. Narimatsu, N. Gokon, S. Tsubouchi, M. Hatakeyama, S. Sakamoto, N. Hanyu, A. Sandhu, H. Kawaguchi, M. Abe, H. Handa, *Colloids Surf., B* **2008**, *64*, 162. b) S. Sakamoto, Y. Kabe, M. Hatakeyama, Y. Yamaguchi, H. Handa, *Chem. Rec.* **2009**, *9*, 66.
27 a) N. Kanoh, K. Honda, S. Simizu, M. Muroi, H. Osada, *Angew. Chem., Int. Ed.* **2005**, *44*, 3559. b) N. Kanoh, S. Kumashiro, S. Simizu, Y. Kondoh, S. Hatakeyama, H. Tashiro, H. Osada, *Angew. Chem., Int. Ed.* **2003**, *42*, 5584.
28 S.-i. Sato, Y. Kwon, S. Kamisuki, N. Srivastava, Q. Mao, Y. Kawazoe, M. Uesugi, *J. Am. Chem. Soc.* **2007**, *129*, 873.
29 M. Ueda, Y. Manabe, Y. Otsuka, N. Kanzawa, *Chem.—Asian J.* **2011**, *6*, 3286; G. Yang, Y. Ishimaru, M. Ueda, unpublished result.
30 a) E. J. Licitra, J. O. Liu, *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 12817. b) C. Chidley, H. Haruki, M. G. Pedersen, E. Muller, K. Johnsson, *Nat. Chem. Biol.* **2011**, *7*, 375.
31 For example: A. W. G. Burgett, T. B. Poulsen, K. Wangkanont, D. R. Anderson, C. Kikuchi, K. Shimada, S. Okubo, K. C. Fortner, Y. Mimaki, M. Kuroda, J. P. Murphy, D. J. Schwalb, E. C. Petrella, I. Cornella-Taracido, M. Schirle, J. A. Tallarico, M. D. Shair, *Nat. Chem. Biol.* **2011**, *7*, 639.
32 For example: a) S. Nishimura, Y. Arita, M. Honda, K. Iwamoto, A. Matsuyama, A. Shirai, H. Kawasaki, H. Kakeya, T. Kobayashi, S. Matsunaga, M. Yoshida, *Nat. Chem. Biol.* **2010**, *6*, 519. b) Y. Kotake, K. Sagane, T. Owa, Y. Mimori-Kiyosue, H. Shimizu, M. Uesugi, Y. Ishihama, M. Iwata, Y. Mizui, *Nat. Chem. Biol.* **2007**, *3*, 570.
33 T. Ito, H. Ando, T. Suzuki, T. Ogura, K. Hotta, Y. Imamura, Y. Yamaguchi, H. Handa, *Science* **2010**, *327*, 1345.
34 For example: M. Kawatani, H. Okumura, K. Honda, N. Kanoh, M. Muroi, N. Dohmae, M. Takami, M. Kitagawa, Y. Futamura, M. Imoto, H. Osada, *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 11691.
35 B. A. Gilbert, R. R. Rando, *J. Am. Chem. Soc.* **1995**, *117*, 8061; Y. Hatanaka, M. Hashimoto, K. I.-P. J. Hidari, Y. Sanai, Y. Nagai, Y. Kanaoka, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2859.
36 T. Tamura, T. Terada, A. Tanaka, *Bioconjugate Chem.* **2003**, *14*, 1222.
37 L. Dubinsky, B. P. Krom, M. M. Meijler, *Bioorg. Med. Chem.* **2012**, *20*, 554; J. Das, *Chem. Rev.* **2011**, *111*, 4405.
38 C. M. Mendel, D. B. Mendel, *Biochem. J.* **1985**, *228*, 269.
39 Y. Nakamura, R. Miyatake, M. Ueda, *Angew. Chem., Int. Ed.* **2008**, *47*, 7289.
40 S.-i. Sato, A. Murata, T. Orihara, T. Shirakawa, K. Suenaga, H. Kigoshi, M. Uesugi, *Chem. Biol.* **2011**, *18*, 131.
41 H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
42 C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057.
43 I. Kii, A. Shiraishi, T. Hiramatsu, T. Matsushita, H. Uekusa, S. Yoshida, M. Yamamoto, A. Kudo, M. Hagiwara, T. Hosoya, *Org. Biomol. Chem.* **2010**, *8*, 4051.
44 S. Yoshida, A. Shiraishi, K. Kanno, T. Matsushita, K. Johmoto, H. Uekusa, T. Hosoya, *Sci. Rep.* **2011**, *1*, Article number 82.
45 Y. Hatanaka, M. Hashimoto, H. Kurihara, H. Nakayama, Y. Kaneoka, *J. Org. Chem.* **1994**, *59*, 383.
46 Y. Hatanaka, Y. Sadakane, *Curr. Top. Med. Chem.* **2002**, *2*, 271.
47 G. Dorman, G. D. Prestwich, *Biochemistry* **1994**, *33*, 5661.
48 G. D. Prestwich, G. Dormán, J. T. Eliott, D. M. Marecak, A. Chaudhary, *Photochem. Photobiol.* **1997**, *65*, 222.
49 E. Weerapana, G. M. Simon, B. F. Cravatt, *Nat. Chem. Biol.* **2008**, *4*, 405.
50 S. Tsukiji, M. Miyagawa, Y. Takaoka, T. Tamura, I. Hamachi, *Nat. Chem. Biol.* **2009**, *5*, 341.
51 S. Cottier, T. Mönig, Z. Wang, J. Svoboda, W. Boland, M. Kaiser, E. Kombrink, *Front. Plant Sci.* **2011**, *2*, Article number 101.
52 C. Laggner, D. Kokel, V. Setola, A. Tolia, H. Lin, J. J. Irwin, M. J. Keiser, C. Y. J. Cheung, D. L. Minor, Jr., B. L. Roth,

R. T. Peterson, B. K. Shoichet, *Nat. Chem. Biol.* **2012**, *8*, 144.
53 H. Yamakoshi, K. Dodo, M. Okada, J. Ando, A. Palonpon, K. Fujita, S. Kawata, M. Sodeoka, *J. Am. Chem. Soc.* **2011**, *133*, 6102.
54 A. Santner, L. I. A. Calderon-Villalobos, M. Estelle, *Nat. Chem. Biol.* **2009**, *5*, 301.
55 A. Santner, M. Estelle, *Nature* **2009**, *459*, 1071, and many references cited therein.
56 T. Sakurai, T. Nakagawa, H. Mitsuno, H. Mori, Y. Endo, S. Tanoue, Y. Yasukochi, K. Touhara, T. Nishioka, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 16653.
57 T. Nakagawa, T. Sakurai, T. Nishioka, K. Touhara, *Science* **2005**, *307*, 1638.
58 K. Ozaki, M. Ryuda, A. Yamada, A. Utoguchi, H. Ishimoto, D. Calas, F. Marion-Poll, T. Tanimura, H. Yoshikawa, *Nat. Commun.* **2011**, *2*, Article number 542.
59 a) A. R. Schneekloth, M. Pucheault, H. S. Tae, C. M. Crews, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5904. b) J. S. Schneekloth, Jr., C. M. Crews, *ChemBioChem* **2005**, *6*, 40.
60 a) K. Cyrus, M. Wehenkel, E.-Y. Choi, H. Swanson, K.-B. Kim, *ChemBioChem* **2010**, *11*, 1531. b) K. Cyrus, M. Wehenkel, E.-Y. Choi, H. Lee, H. Swanson, K.-B. Kim, *ChemMedChem* **2010**, *5*, 979.
61 Y. Itoh, M. Ishikawa, M. Naito, Y. Hashimoto, *J. Am. Chem. Soc.* **2010**, *132*, 5820.
62 A. Suzuki, K. Tsumura, T. Tsuzuki, S. Matsumura, K. Toshima, *Chem. Commun.* **2007**, 4260.
63 a) D. Takahashi, S. Hirono, C. Hayashi, M. Igarashi, Y. Nishimura, K. Toshima, *Angew. Chem., Int. Ed.* **2010**, *49*, 10096. b) Y. Ishida, S. Tanimoto, D. Takahashi, K. Toshima, *MedChemComm* **2010**, *1*, 212. c) S. Tanimoto, S. Sakai, S. Matsumura, D. Takahashi, K. Toshima, *Chem. Commun.* **2008**, 5767. d) S. Tanimoto, S. Matsumura, K. Toshima, *Chem. Commun.* **2008**, 3678.
64 Y. Nakamura, A. Mithöfer, E. Kombrink, W. Boland, S. Hamamoto, N. Uozumi, K. Tohma, M. Ueda, *Plant Physiol.* **2011**, *155*, 1226.
65 B. Thines, L. Katsir, M. Melotto, Y. Niu, A. Mandaokar, G. Liu, K. Nomura, S. Y. He, G. A. Howe, J. Browse, *Nature* **2007**, *448*, 661.
66 A. Chini, S. Fonseca, G. Fernández, B. Adie, J. M. Chico, O. Lorenzo, G. García-Casado, I. López-Vidriero, F. M. Lozano, M. R. Ponce, J. L. Micol, R. Solano, *Nature* **2007**, *448*, 666.
67 L. B. Sheard, X. Tan, H. Mao, J. Withers, G. Ben-Nissan, T. R. Hinds, Y. Kobayashi, F.-F. Hsu, M. Sharon, J. Browse, S. Y. He, J. Rizo, G. A. Howe, N. Zheng, *Nature* **2010**, *468*, 400.
68 M. Isobe, Abstract of 17th Banyu-Fukuoka-Symposium, **2007**.
69 a) Y. Nakagawa, R. C. Yanagita, N. Hamada, A. Murakami, H. Takahashi, N. Saito, H. Nagai, K. Irie, *J. Am. Chem. Soc.* **2009**, *131*, 7573. b) R. C. Yanagita, H. Kamachi, K. Tanaka, A. Murakami, Y. Nakagawa, H. Tokuda, H. Nagai, K. Irie, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6064.
70 J. P. Arcoleo, I. B. Weinstein, *Carcinogenesis* **1985**, *6*, 213.

