Protein Trans-Splicing of an Atypical Split Intein Showing Structural Flexibility and Cross-Reactivity

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Abstract

Inteins catalyze a protein splicing reaction to excise the intein from a precursor protein and join the flanking sequences (exteins) with a peptide bond. In a split intein, the intein fragments (IN and IC) can reassemble non-covalently to catalyze a trans-splicing reaction that joins the exteins from separate polypeptides. An atypical split intein having a very small IN and a large IC is particularly useful for joining synthetic peptides with recombinant proteins, which can be a generally useful method of introducing site-specific chemical labeling or modifications into proteins. However, a large IC derived from an Ssp DnaX intein was found recently to undergo spontaneous C-cleavage, which raised questions regarding its structure-function and ability to trans-splice. Here, we show that this IC could undergo trans-splicing in the presence of IN, and the trans-splicing activity completely suppressed the C-cleavage activity. We also found that this IC could trans-splice with small IN sequences derived from two other inteins, showing a cross-reactivity of this atypical split intein. Furthermore, we found that this IC could trans-splice even when the IN sequence was embedded in a nearly complete intein sequence, suggesting that the small IN could project out of the central pocket of the intein to become accessible to the IC. Overall, these findings uncovered a new atypical split intein that can be valuable for peptide-protein trans-splicing, and they also revealed an interesting structural flexibility and cross-reactivity at the active site of this intein.

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Introduction

Inteins are internal protein sequences that can catalyze a protein splicing reaction to excise themselves from a precursor protein and at the same time join the flanking sequences (N- and C-exteins) with a peptide bond [1,2]. The catalytic mechanism of protein splicing typically consists of four steps [2,3]: 1) an N-S or N-O acyl rearrangement at the upstream splicing junction replaces the peptide bond with an ester bond; 2) a transesterification reaction transfers the N-extein from the upstream splice junction to the downstream splice junction; 3) an asparagine cyclization at the C-terminus of the intein breaks the peptide bond, which separates the intein from the exteins; and 4) a S-N or O-N acyl rearrangement forms a peptide bond joining the N- and C-exteins. Inteins can sometimes catalyze protein cleavage at their N- or C-termini when the splicing mechanism is disrupted [3]. For example, when step 1 of the splicing mechanism is blocked or missing, step 3 may still occur and break the peptide bond at the C-terminus of the intein, in what is termed C-cleavage. Whereas different inteins show low levels of similarity at the amino acid sequence level, the crystal structures of different inteins (the protein splicing domain) appear very similar [4,5,6,7]. The splicing domains of inteins are typically shaped like a flattened disk consisting of ~12 β-strands, with the N- and C-terminal parts of the intein folded into a centrally located catalytic pocket.

In a split intein, the intein sequence is broken into two separate fragments, and these intein fragments (IN and IC) can reassemble non-covalently to catalyze a protein trans-splicing reaction [8,9]. This reaction takes place between two separate polypeptides, with one polypeptide consisting of an N-extein fused to the IN and the other polypeptide consisting of the IC fused to a C-extein. During trans-splicing, the IN and IC are excised, while the N- and C-exteins are concomitantly joined with a peptide bond. Protein-protein trans-splicing using split inteins has proven to be a useful technology with a wide range of applications. Examples include production of cytotoxic proteins that cannot be expressed in a single piece [10,11], segmental isotope labeling of proteins for NMR studies [12], and a gene therapy procedure using split genes [13]. For peptide-protein trans-splicing, a non-canonical split intein (Ssp DnaB S1) consisting of a small IN and a large IC has been engineered [14]. This atypical split intein is particularly suitable for splicing synthetic peptides onto the N-terminus of recombinant proteins, because the extremely small IN (11 aa long) can be more readily produced together with a small N-extein through chemical synthesis. Because the chemically synthesized N-extein can potentially contain any desired chemical group, this atypical split intein has been used successfully in adding fluorescent labels to the N-terminus of recombinant proteins in vitro [15] and on the surface of live mammalian cells [16].
Further development of the intein-based method of protein site-specific modification or labeling is of great interest for protein research and engineering. For example, fluorescent or isotope labels can be useful for studying cellular location and trafficking of proteins, and chemical modifications (e.g. unnatural amino acids) can aid studies of protein’s structure-function relationship. Standard chemical methods often produce mixed populations of the modified protein, because such methods usually target certain amino acid side chains (thiols, carboxyls, amines) that may exist at multiple locations in the protein [17]. Other methods have been developed for site-specific protein modifications with limited success. Certain polypeptide tags have been used in recombinant proteins to attach a chemical group through an enzymatic reaction (e.g. [18]), but the tag remains incorporated in the modified protein and may interfere with protein function. Specially engineered tRNA charging systems have been used to add unnatural amino acids to proteins during translation [19], but it is difficult or impossible to engineer special tRNA charging systems for every desired unnatural amino acid or chemical modification. Intein-based protein-peptide trans-splicing is a newer and potentially more useful method for site-specific protein modifications [20,21,22], because it does not leave a large tag in the modified protein and may be used generally with any chemical moieties on the chemically synthesized extein peptide. To further develop this intein-based method, it is important to find new and atypical split inteins for the peptide-protein trans-splicing, because different inteins may exhibit different splicing efficiencies with different exteins [23,24,25,26]. In a recent such study, however, a large C-intein (IC) derived from the Sph DnaX intein (a natural intein in DnaX protein of Synechocystis sp. PCC6803) was found to undergo spontaneous C-cleavage [27], which is unlike the similarly constructed IC of the Sph DnaB intein (a natural intein in DnaB protein of Synechocystis sp. PCC6803). This C-cleavage activity was unexpected, because the large IC without IN was thought to have a structural hole in the catalytic pocket of the intein, based on the predicted structure of the intein. This surprise finding raised interesting questions regarding the structure and function of this intein, namely whether it can still catalyze protein trans-splicing in the presence of the IN and, if so, whether its catalytic pocket has unusual structural flexibilities that are not apparent from intein crystal structures.

In this study, we initially found that the unusual IC of the Sph DnaX intein could undergo protein trans-splicing with the small IN on another protein, and this trans-splicing activity completely predominated over the C-cleavage activity. The IC could also trans-splice with small IN sequences derived from other different inteins, which revealed for the first time a cross-reactivity of atypical split inteins. We also found that the small IN could be replaced functionally by a nearly complete intein containing the IN. These findings not only generated a new and second atypical split intein suitable for trans-splicing peptides onto the N-terminus of proteins, they also have interesting implications for the structure-function of this atypical intein and perhaps also other inteins. We suggest that the centrally located catalytic pocket of the intein might undergo reversible transitions between an open state for the trans-splicing function and a closed state for the C-cleavage function, and this structural flexibility might permit the IN part of the intein to swing out of the central pocket of the intein.

Results

First we determined whether the 139-aa C-intein (IC) of the Sph DnaX intein could undergo protein trans-splicing when the missing 11-aa N-terminal part (N-intein or IN) was provided in trans, because the IC alone had been found to undergo spontaneous C-cleavage [27]. As illustrated in Figure 1A, a maltose binding protein (M) and a thioredoxin protein (T) were used as the N-extein and C-extein, respectively, so that a trans-splicing reaction would join these two exteins to form the splicing product MT. As shown in Figure 1B, the splicing product MT was produced both in vivo when the two precursor proteins (MIN and ICT) were co-expressed in E. coli and in vitro when the purified precursor proteins were incubated together in a test tube.

Interestingly, no C-cleavage activity was detected under these conditions, as indicated by the absence of the cleavage product T. The precursor and product proteins were identified by their predicted sizes and specific recognition of an anti-thioredoxin (anti-T) antibody through Western blotting. For the in vitro analysis in E. coli cells, only the splicing product MT protein was detected using anti-T antibody, indicating that the precursor protein IC-T had trans-spliced completely to form the MT protein. For the in vitro analysis, the purified precursor protein MIN was added in excess to the precursor protein IC-T to drive the trans-splicing reaction to greater completion. In producing the IC-T protein alone in E. coli, a significant amount of spontaneous C-cleavage occurred as reported previously [27]. In the purification of IC-T using an affinity tag (hexahistidine) contained in the IC, the cleavage product IC was co-purified with the remaining IC-T in the purified sample, while the cleavage product T lacked the hexahistidine tag and was absent in the purified sample. The purified IC-T protein did not show new C-cleavage during subsequent co-incubation with the MIN protein for trans-splicing, as indicated by the absence of any new formation of the C-cleavage products IC and T. Under the in vivo conditions used, approximately 85% of the IC-T protein was trans-spliced to form the MT protein after 20 hours of incubation at room temperature (Figure 1B). We also tested shorter reaction times and different temperatures (Figure 1C). The efficiency of trans-splicing was nearly identical at four tested temperatures (4, 25, and 37°C, and on ice) after 24 hours of reaction. With a shorter reaction time of 15 minutes, the efficiency of trans-splicing was a little lower at 4°C and significantly lower on ice.

We then investigated whether the trans-splicing reaction could still occur when the small IN is embedded in a near complete intein. As illustrated in Figure 2A, the intein fragment INL was designed to contain the N-terminal 144-aa sequence of the 150-aa Sph DnaX intein that lacked the C-terminal 6 aa of the intein, to prevent possible self-cleavage or cis-splicing. This INL was found to trans-splice efficiently with IC in vivo at three different temperatures (4, 25, and 37°C), where M and T were the exteins (Figure 2B). Because the 144-aa INL consists of the small (11-aa) IN plus other parts of the intein, we asked whether the other parts of the intein also participated in the trans-splicing reaction. To answer this question, a double mutation (TXHH to AXXA) was introduced in the Block B motif of the intein, because this conserved intein sequence motif is outside the 11-aa IN and known to be functionally important in inteins [25]. As seen in Figure 2C, mutating the Block B motif of IC (resulting in ICm) destroyed its ability to trans-splice with IN, as expected. In contrast, mutating the Block B motif of INL (resulting in INLm) did not affect its ability to trans-splice with IC, indicating that the Block B motif in INL did not participate in the reaction. We also tested mutated C-intein (ICNL) in a combination with the non-mutated version of INL, and found that latter could compensate for the mutated Block B motif in the former for the trans-splicing reaction. When the Block B motif was mutated in both INL (resulting in INLm) and IC (resulting in ICm), the trans-splicing reaction was abolished, as expected.

To further explore the structural flexibility and versatility of this atypical split intein, we asked whether the large IC could trans-splice...
with small IN sequences derived from other inteins. The 12-aa INRB was derived from the N-terminus of the RmaDnaB intein (a natural intein in DnaB protein of *Rhodothermus marinus*) [28] that is highly similar to the SspDnaB intein from which the first atypical split intein was derived [13]. The 12-aa INSG was derived from the N-terminus of the SspGyrB intein (a natural intein in GyrB protein of *Synechocystis* sp. PCC6803) [29]. As shown in Figure 3A, the INRB sequence is 41% identical (58% similar) to the IN sequence, and the INSG sequence is 50% identical (75% similar) to the IN sequence. Under *in vivo* conditions in *E. coli*, both INRB and INSG trans-spliced efficiently with IC, as indicated by the accumulation of the splicing product MT but not precursor protein ICT (Figure 3B). This may indicate an inefficient use of INRB under the *in vitro* conditions used, although an efficient use of INRB was seen in *E. coli* cells. With both INRB and INSG, the precursor protein ICT underwent a small amount of C-cleavage, as indicated by the accumulation of the C-cleavage product T.

**Discussion**

The atypical *Ssp* DnaX intein is found, for the first time, to be capable of protein trans-splicing, despite the fact that its large C-intein (Ic) had been known to undergo spontaneous C-cleavage.
This finding has interesting implications on the structure-function of inteins’ active site. Previously the IC part of this intein was found to undergo spontaneous C-cleavage in the absence of IN [27], which was quite unexpected and unlike other inteins. The highly conserved crystal structures of inteins predict that the N- and C-terminal parts of an intein are located in a central catalytic pocket [5,6,7], as illustrated by a computer modeling of the Ssp DnaX intein shown in Figure 4A. The 11-aa IN sequence forms two small β-strands named β1 and β2, with β1 being buried deep inside the intein structure. Without IIN, the IC structure has been predicted to have a structural void (hole) in its catalytic pocket [27], as illustrated in Figure 4C. A similar prediction has also been made for IC of the Ssp DnaB intein [30], where the hole was thought to be a docking place for the IN to trigger a C-cleavage reaction. To explain why the IC of Ssp DnaX intein (but not of Ssp DnaB intein) could undergo spontaneous C-cleavage in the absence of IIN, it was...

Figure 2. Effects of IIN size and Block B mutation on trans-splicing. A. Schematic illustration of experimental designs. IINL is the N-terminal 144-aa sequence of the Ssp DnaX intein. IINLm and IICm are the same as IINL and IIC, respectively, except that the conserved Block B sequence of the intein was mutated from TXXH to AXXA. Others are the same as in Figure 1A. B. Trans-splicing of IC-T with MINL. A mixture of the two precursor proteins was incubated at the specified temperatures for 20 hours to allow reaction, with the protein bands visualized by Western blotting using anti-T antibody. C. Trans-splicing of different precursor protein pairs listed in panel A. Each pair of precursor proteins was incubated together at room temperature for 20 hours, with the protein bands visualized by Western blotting using anti-T antibody. Names of only intein parts of the precursor proteins are specified on the top of the panel.

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Figure 3. Cross-reactivity of IC with IN from different inteins. A. Comparison of the amino acid sequences of IIN from Ssp DnaX intein (IIN), Rma DnaB intein (IINRB), and Ssp GyrB intein (IINSG). Identical and similar residues are marked with a | and a :, respectively. A gap (represented with a -) is introduced in the IIN sequence to maximize the sequence alignment. B. Analysis of trans-splicing reactions between IIN and INRB (or INSG) as specified on top. For in vivo analysis, MINRB (or MINSG) and ICT proteins were co-expressed in E. coli cells, and total cellular proteins were analyzed by Western blotting using an anti-thioredoxin (Anti-T) antibody. For in vitro analysis, purified MINRB (or MINSG) and ICT proteins were co-incubated at room temperature for 20 hours, and the reaction products were analyzed by Western blotting as above. Positions are marked for the precursor ICT, splicing product MT, and C-cleavage product T. Size markers are shown on the left.

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Furthermore, we discovered cross-reactivity between IC of the replaced with INL, where INL is a near complete intein containing Ssp atypical splicing. We suggest that the hole of IC may exist in two equilibrium states: an open state (hole is open) allowing the docking of IC for trans-splicing, and a closed state (hole is closed) allowing spontaneous C-cleavage without IN. Our findings also indicate that the open state predominates, because the trans-splicing reaction completely suppressed the C-cleavage reaction when IC was present. Our suggestion is consistent with an earlier study of the Ssp DnaB intein, where the horseshoe-like structure of the large IC was suggested to open up and clamp onto the small IN [30].

The above suggestion is further supported by our finding that the trans-splicing reaction could occur when the small IN was replaced with INL, where INL is a near complete intein containing the IN. The IN part of INL must have participated in the trans-splicing reaction, whereas the remaining part of INL (at least the conserved Block B motif) apparently did not participate in the reaction, because a mutation in the Block B motif of INL did not prevent trans-splicing. To participate in the trans-splicing reaction, the IN part of INL needs to move out from its buried position in INL. (Figure 4D), before it can dock into the catalytic pocket (open hole) of IC for trans-splicing. This suggests that the INL structure is able to open up in order for the IN part to ‘swing out’ to an exposed position. Consistent with this suggestion, a mutated C-intein (ICm) trans-spliced with INL, suggesting that the ICm structure could open up to allow its C-terminal part to ‘swing out’ to an exposed position for participation in the trans-splicing reaction. Furthermore, we discovered cross-reactivity between IC of the atypical Ssp DnaX split intein and the small IN from two other inteins, which is the first suggestion of cross-reactivity for such atypical split inteins. Considering that the IN of the other inteins is only 40–50% identical to the native IN in amino acid sequence, it is interesting that the non-native IN can correctly dock into the structural hole of the IC and catalyze the trans-splicing reaction. Overall, these findings revealed an interesting structural flexibility at or near the catalytic pocket of inteins, which can have significant implications on future engineering of split inteins for peptide-protein or protein-protein trans-splicing for various applications.

The trans-splicing function of this atypical Ssp DnaX split intein also makes a significant addition to the intein-based toolbox for general uses. Previously only the Ssp DnaB intein has been engineered into such an atypical split intein, and was named the S1 split intein [14]. Unlike other forms of split inteins, the S1 split intein has an extremely small IN and is therefore particularly useful for splicing synthetic peptides onto the N-terminus of target proteins. The synthetic peptide can easily accommodate the 11-aa IN plus a small N-extein to be spliced onto the N-terminus of a target protein, with the target protein becoming a fusion protein containing the IC. This peptide-protein trans-splicing is useful for site-specific labeling or modifications of proteins, because the synthetic N-extein may be engineered to carry a variety of chemical moieties, including fluorescent groups, modified or unnatural amino acids, and drug molecules, as long as the chemical moiety does not block trans-splicing. Finding and understanding new S1 split inteins, as we have done in this study, is important for wide uses of this peptide-protein trans-splicing method, because different inteins have been known to splice differently when used on different target proteins [25,31]. It is impressive that this new S1 split intein could perform the trans-splicing reaction at temperatures ranging from ~1°C (on ice) to 37°C, although the reaction speed was somewhat lower at 1–4°C temperatures. This temperature tolerance may be due to the fact that this intein was derived from a natural intein found in a cyanobacterium (Syenochocystis sp. PCC6803) that lives under a wide range of environmental temperatures. This robust nature of the S1 split intein can be an advantage in practical applications where one may need to achieve trans-splicing under low temperatures. Our finding of cross-reactivity between the IC of the atypical Ssp DnaX split intein and the small IN from two other inteins also has interesting implications. On the one hand, it permits different choices for the IN for doing peptide-protein trans-splicing and suggests that the IN sequence may tolerate many sequence changes, which can be useful information for designing and producing synthetic peptides containing IN. On the other hand, two atypical split inteins may not be used together in a mixed system to achieve labeling or modification of two different target proteins in a protein-specific manner.

Materials and Methods

Plasmid Construction

Plasmid pMSX-S1 for in vivo experiments was constructed as described previously [14], in which the two open reading frames expressing the IN and IC proteins were separated by a spacer sequence. A restriction enzyme cutting site All II was introduced at the split site. To construct plasmid pMSX-S1Nexpressing the IN protein alone, a DNA fragment between All II and Hind III sites was deleted from plasmid pMSX-S1. Plasmid pMSX-S1Cexpressing the IC protein alone was from [27]. Plasmid pMSX-S1NL expressing the IN protein was constructed by replacing the IN coding sequence in pMSX-S1N with the first 141 codons of the Sip DnaX intein [32], which used standard recombinant DNA methods including PCR, DNA cutting (XhoI-All II), and ligation. Plasmid pMRB-S1N expressing the IN protein and plasmid pMSG-S1N expressing the MSG protein were constructed by replacing the IN coding sequence in pMSX-S1N with the first 12 codons of the Rma DnaB intein [28] and Sip GyrB intein [29].
respectively. To construct pMRBSX-S1, pMSGSX-S1, the IN coding sequence between XhoI and Afl II in pMSX-S1 was replaced with INRB or INSG sequence respectively. To construct plasmid pmMSX-S1C (or pmMSX-S1NL), expressing the IcT or InT protein, site-directed mutations were introduced into plasmid pMSX-S1C (or pMSX-S1NL), using a standard method of inverse PCR.

Protein Expression, Purification and in vitro Reactions
Plasmids pMSX-S1, pMSX-S1N, pMSX-S1NL, pMRBSX-S1, pMSGX-S1NL and pmMSX-S1NL were each transformed into *E. coli* strain BL21(DE3), while, plasmids pMSX-S1C and pmMSX-S1C were each transformed into *Esherichia coli* BL21(DE3). All plasmids were used according to the manufacturer’s instructions (New England Biolabs). To purify the Ict and InT proteins containing the maltose binding protein, amylose resin was used according to the manufacturer’s instructions (NE Biolabs). To purify the IC and ICmT proteins containing a hexahistidine tag, Ni-NTA resin (QIAGEN) was used according to the manufacturer’s instructions. For in vitro trans-splicing or cleavage reactions, the specified precursor proteins were mixed and incubated under specified conditions, with 1 mM DTT added to all in vitro reactions. Western blotting used an anti-thioredoxin (anti-T) antibody (Invitrogen) and the Enhanced Chem-luminescence detection kit (GE Healthcare), all according to the manufacturer’s instructions.

Computational Simulations of Intein Structure
A simulated three-dimensional structure of *S* pDanX mini-intein was obtained by using the fully automated homology-modeling pipeline SWISS-MODEL [33,34]. The intein amino acid sequence [27] was uploaded to the Automatic Modeling Workspace (http://swissmodel.expasy.org/workspace), the crystal structure of *S* pDnaB mini-intein was selected automatically as a closest template by homology, and structural models of *S* pDanX mini-intein were generated and presented in NewCartoon style.

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Author Contributions
Conceived and designed the experiments: XQL. Performed the experiments: HS. Analyzed the data: HS QM XQL. Contributed reagents/materials/analysis tools: QM XQL. Wrote the paper: HS XQL.

References
1. Perler FB, Davis EO, Doen GE, Gumbleo JS, Jack WE, et al. (1994) Protein splicing elements: inteins and exteins: definition of terms and recommended nomenclature. Nucleic Acids Res 22: 1125–1127.
2. Perler FB (2005) Protein splicing mechanisms and applications. IURMB Life 57: 469–476.
3. Xu MQ, Perler FB (1996) The mechanism of protein splicing and its modulation by mutation. Emb J 15: 5146–5153.
4. Klabeude T, Sharma S, Toleni A, Jacobs WR, Jr., Sarcchetti JC (1998) Crystal structure of GyrA intein from Mycobacterium xenopi reveals structural basis of protein splicing. Nat Struct Biol 5: 31–36.
5. Ichiyangak K, Ishino Y, Aoyashii M, Komori K, Morikawa K (2000) Crystal structure of an archael intein-encoded homing endonuclease PI-Pf. J Mol Biol 300: 829–901.
6. Hall TM, Porter JA, Young KE, Koonin EV, Beachy PA, et al. (1997) Crystal structure of a Hedeghog autoprocessing domain: homology between Hedeghog and self-splicing proteins. Cell 91: 85–97.
7. Ding Y, Xu MQ, Ghoss I, Chen X, Ferrandon S, et al. (2003) Crystal structure of a mini-intein reveals a conserved catalytic module involved in side chain cyclization of asparagine during protein splicing. J Biol Chem 278: 39133–39142.
8. Wu H, Hu Z, Liu XQ (1998) Protein trans-splicing by a split intein encoded in a split Dna gene of Synechospyrus sp. PPC6003. Proc Natl Acad Sci U S A 95: 9226–9231.
9. Evans TC, Jr., Martin D, Kolly R, Panne D, Xu L, et al. (2000) Protein trans-splicing and cyclization by a naturally split intein from the dnaE gene of Synechospyrus species PPC6003. J Biol Chem 275: 9091–9094.
10. Evans TC, Jr., Benner J, Xu MQ (1998) Semisynthesis of cysteine proteins using a modified protein splicing element. Protein Sci 7: 2256–2264.
11. Wu W, Wood DW, Belfort G, Derhaviey B, Belfort M (2002) Intein-mediated purification of cystotic endonuclease I-Tevl by inserctional inactivation and pH-controllable splicing. Nucleic Acids Res 30: 4704–4711.
12. Toohla YM, Yamazaki TO, Natsuko Oda, Yoshimasa Kyogoku, Koski Urgaki, Nobutoshi Isso, Yoshinumi Ishino, Haruki Nakamura (1998) Segmental isotope labeling for protein NMR using peptide splicing. Journen American Chemical Society 120: 2.
13. Li J, Sun W, Wang B, Xiao X, Liu XQ (2008) Protein trans-splicing as a means for viral vector-mediated in vivo gene therapy. Hum Gene Ther 19: 958–964.
14. Sun W, Yang J, Liu XQ (2004) Synthetic two-piece and three-piece split inteins for protein trans-splicing. J Biol Chem 279: 35281–35286.
15. Ludwig C, Pleff M, Linne U, Mootse HD (2006) Ligation of a synthetic peptide to the N terminus of a recombinant protein using semisynthetic protein trans-splicing. Angew Chem Int Ed Engl 45: 5218–5221.
16. Audo T, Tsukui S, Tanaka T, Hagamine T (2007) Construction of a small-molecule-integrated semisynthetic split intein for in vivo protein ligation. Chem Commun (Camb): 1945–1947.
17. Gilmore JM, Scheck RA, Esah-Kalphi AP, Joshi NS, Francis MB (2006) N-terminal protein modification through a biomimetic transamination reaction. Angew Chem Int Ed Engl 45: 5307–5312.
18. Lin CW, Ting SY (2006) Transglutaminase-catalyzed site-specific conjugation of small-molecule probes to proteins in vitro and on the surface of living cells. J Am Chem Soc 128: 4542–4543.
19. Xie J, Schultz PG (2006) A chemical toolkit for proteins—an expanded genetic code. Nat Rev Mol Cell Biol 7: 775–782.
20. Muir TW (2003) Semisynthesis of proteins by expressed protein ligation. Annu Rev Biochem 72: 294–309.
21. Tan LP, Yao SQ (2005) Intein-mediated, in vitro and in vivo protein modifications with small molecules. Protein Pept Lett 12: 769–775.
22. Kala J, Raines RT (2006) Reactivity of intein thiocysteines: appending a functional group to a protein. Chem Commun 7: 1373–1383.
23. Banati G, Callahan BP, Stanger MJ, Belfort G, Belfort M (2009) Modulation of intein activity by its neighboring extein substrates. Proc Natl Acad Sci U S A 106: 11002–11010.
24. Iwai H, Suge S, Je J, Tarn PH (2006) Highly efficient protein trans-splicing by a naturally split DnaB intein from Nostoc punctiforme. FEBS Lett 580: 1853–1858.
25. Noren CJ, Wang J, Perler FB (2000) Dissecting the Chemistry of Protein Splicing and its Applications. Angew Chem Int Ed Engl 39: 450–466.
26. Appleye-Tague JL, Thiel IV, Wang Y, Wang Y, Mootse HD, et al. (2011) Highly efficient and more general cis- and trans-splicing inteins through sequential directed evolution. J Biol Chem 286: 34440–34447.
27. Qu X, Meng Q, Liu XQ (2011) Spontaneous C-cleavage of a mini-intein without its conserved N-terminal motif A. FEBS Lett 586: 2513–2518.
28. Liu XQ, Hu Z (1997) A Dnab intein in Rhodothermus marinus: indication of recent intein homing across remotely related organisms. Proc Natl Acad Sci U S A 94: 7851–7856.
29. Gorbalenya AE (1998) Non-canonical inteins. Nucleic Acids Res 26: 1741–1748.
30. Volkmann G, Sun W, Liu XQ (2009) Controllable protein cleavages through intein fragment complementation. Protein Sci 18: 2393–2402.
31. Paulus H (2001) Inteins as enzymes. Biochim Biophys Acta 1481: 119–129.
32. Liu XQ, Hu Z (1997) Identification and characterization of a cyanoabacterial DnaX intein. FEBS Lett 408: 311–314.
33. Kiefer F, Arnold K, Kunzli M, Bordoli L, Schwede T, et al. (2004) The SWISS-MODEL Repository and associated resources. Nucleic Acids Res 33: D338–340.
34. Kopp J, Schwede T (2004) The SWISS-MODEL Repository: An annotated three-dimensional structure database. Nucleic Acids Res 33: D222–D225.