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Internal Disulfide Bond Acts as a Switch for Intein Activity

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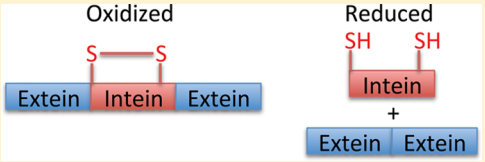
Supporting Information

ABSTRACT: Inteins are intervening polypeptides that catalyze their own removal from flanking exteins, concomitant to the ligation of the exteins. The intein that interrupts the DP2 (large) subunit of DNA polymerase II from *Methanoculleus marisnigri* (*Mma*) can promote protein splicing. However, protein splicing can be prevented or reduced by overexpression under nonreducing conditions because of the formation of a disulfide bond between two internal intein Cys residues. This redox sensitivity leads to differential activity in different strains of *E. coli* as well as in different cell compartments. The redox-dependent control of in vivo protein splicing in an intein derived from an anaerobe that can occupy multiple environments hints at a possible physiological role for protein splicing.



Protein splicing is a self-catalyzed process facilitated by an intein. The intein interrupts two flanking polypeptides, called the N- and C-exteins, and promotes both its own excision and the ligation of these exteins.[1,2]

Protein splicing usually follows a four-step mechanism (Figure 1A). First, the peptide bond linking the N-extein and intein is converted to a linear ester or thioester by nucleophilic attack by the intein's N-terminal Ser or Cys. Next, the N-extein is transferred from the side chain of the first residue of the intein to the side chain of the first residue of the C-extein (Ser, Thr, or Cys) via transesterification. In step 3, cyclization of the conserved C-terminal Asn residue of the intein, coupled to peptide bond cleavage and aminosuccinimide formation, separates the intein from the exteins. Concomitantly, an ester bond links the exteins. Finally, the aminosuccinimide may be hydrolyzed, and the exteins' ester linkage is rapidly converted to a peptide bond.[1,2] If the steps of splicing are poorly coordinated, side reactions can occur. In N-terminal cleavage, the linear or branched ester formed in steps 1 and 2 can be cleaved, uncoupled from splicing. In addition, Asn cyclization uncoupled from splicing can result in C-terminal cleavage.

Protein splicing can be essential for the activity of the flanking proteins.[3,4] It has been suggested that inteins, particularly in association with intervening homing endonuclease domains, are parasitic genetic elements. As such, inteins persist in their host sequence because they have little effect on fitness, are present in highly conserved sites, or are capable of using their associated homing endonuclease to invade inteinless alleles.[5−8] If, instead, inteins are present in host proteins because they play a beneficial role, perhaps by regulating the activity of their flanking exteins, splicing would need to be responsive to a trigger.

Some native inteins, and others modified by molecular engineering, can promote conditional protein splicing. Inteins from thermophilic organisms can be purified as unspliced precursors from overexpression in *E. coli* and induced to splice at high temperature.[9−11] Engineered inteins have been designed to facilitate splicing (or cleavage side reactions) in response to changes in light or protease activity,[12,13] small molecules,[14−16] pH,[17,18] or temperature.[10,19−22] Protein splicing in *trans*, in which the intein is split into two separate fragments, depends on reassociation of these fragments. Split-intein reassociation has been triggered in engineered systems by light or the addition of a small molecule, both in vitro and in vivo.[13,23−28]

Conditional protein splicing also can be triggered by the reduction of a disulfide bond that prevents splicing of the intein. Such disulfide bonds have been engineered between intein and extein Cys residues to control splicing activity.[29−33] Native disulfide bonds that influence activity, either between Cys1 and Cys+1[34,35] or between Cys1 and a Cys in the N-extein,[30] have also been described. Autoprocessing by hedgehog domains, which are similar in structure and sequence to inteins, depends on reduction of a disulfide bond between Cys1 of the processing domain and a conserved downstream Cys.[36]

We report an intein with an in vivo splicing activity that is controlled by the oxidation state of a disulfide bond. Unlike inteins with disulfide bonds between extein and intein Cys residues, this intein has a disulfide bond between Cys residues within the intein. We show that the intein that interrupts the DP2 subunit of DNA polymerase II from *Methanoculleus marisnigri* (the *Mma* PolII intein)[37] can promote protein splicing when overexpressed in *E. coli* BL21(DE3). However, when overexpressed in an *E. coli* strain that promotes disulfide bond formation, we isolate unspliced precursor. This precursor can be induced to undergo N-terminal cleavage upon in vitro

Received: June 11, 2013
Revised: July 30, 2013
Published: August 1, 2013

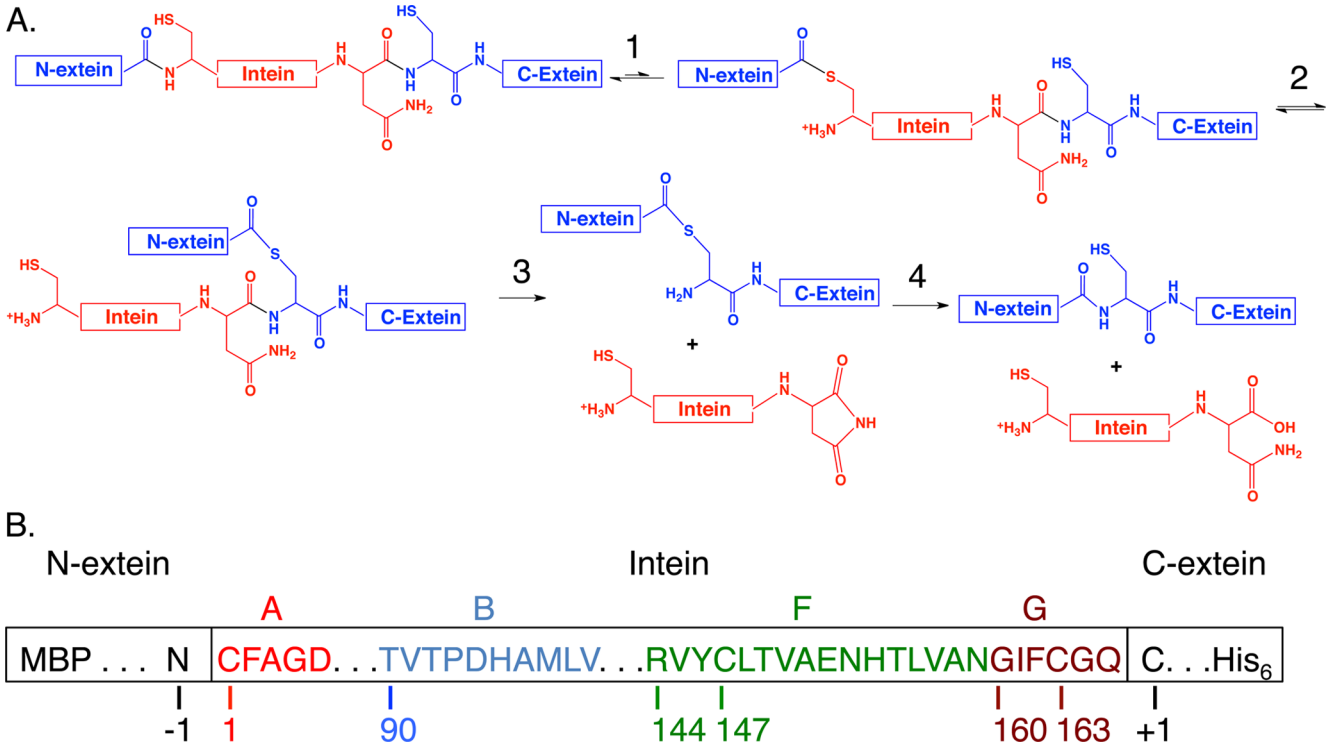


Figure 1. Mechanism of protein splicing and schematic of the *Mma* PolII intein. (A) Overview of canonical mechanism for protein splicing. (B) Schematic of the fusion protein, not to scale. The letters above the boxes indicate the conserved intein sequence blocks, the color-coded residues indicate the sequence, and the numbers below the boxes indicate the numbering scheme. For numbering the intein and C-extein, residue numbers increase downstream with residues 1 and +1 the first residues, respectively. For the N-extein, the final residue is −1, with values proceeding upstream.

incubation with 1,4-dithiothreitol (DTT). Unusual migration on SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), resolvable by reduction, indicates the presence of a disulfide bond in the unspliced precursor. We also show that splicing is dependent on the cellular oxidation state, with differential activity in different *E. coli* strains as well as between the cytoplasm and periplasm of the same strain.

EXPERIMENTAL PROCEDURES

Plasmid Preparation. We created an *E. coli* expression vector for a fusion protein of the *E. coli* maltose binding protein (MBP) to the 10 C-terminal residues of the N-extein, the 165-residue intein, the 6 N-terminal residues of the C-extein, and a poly His tag. Short linkers connect the MBP and His tag to the extein segments. To create this vector, pMIHMma, we amplified the *Mma* PolII gene by PCR from genomic DNA (ATCC, 35101D) with primers MmaU (5′-GGCTACGG-CAGGCCTTTCTTCCACG) and MmaL (5′-GAGGAGC-GAATTCCAGTCCTCGTCGCCGTC). The PCR product was inserted between the *Stu*I and *Eco*RI sites of plasmid pPabPol1His.[11] To facilitate periplasmic expression, we digested pMIHMma with *Stu*I and *Hin*dIII and transferred the intein-containing sequence to pMal-p2x (New England Biolabs) to make pMIHMma-p2x. We created site-directed mutants of the intein with appropriate primers as described.[38] The sequence of the intein was verified by DNA sequencing and was consistent with the NCBI database (accession number ABN58047).

Protein Expression and Purification. To facilitate overexpression under reducing or oxidizing conditions, plasmids were transformed into *E. coli* BL21(DE3) or Origami 2(DE3), respectively (Novagen). We induced expression at midlog phase with 1 mM isopropyl-*β*-D-1-thiogalactopyranoside and incubated the cells with shaking at 20 °C for 16 h.

Overexpressed fusion proteins were purified through their C-terminal His tags. *E. coli* were pelleted by centrifugation at 3000*g*, and pellets were suspended in buffer A (20 mM HEPES, pH 7.5, 500 mM NaCl) supplemented with BugBuster Extraction buffer (Novagen), 12 units/mL benzonase nuclease, 100 *μ*M phenylmethylsulfonyl fluoride, and Protease Inhibitor Cocktail P8849 (Sigma-Aldrich). The cellular extract was either analyzed by Western blot or further purified using a Talon metal affinity resin (Clontech) prewashed with buffer A. After loading the sample, the column was washed with 3 × 10 mL of buffer A supplemented with 10 mM imidazole and 0.1% Tween-20 and eluted with 3 × 500 *μ*L of buffer A supplemented with 200 mM imidazole. Protein concentration was determined by the Bradford method.[39] Proteins were exchanged against buffer A using a Millipore Ultracel 0.5 ml centrifugal filter, 3000 MWCO.

Protein Analysis. To analyze protein splicing by SDS-PAGE, we used precast 4−20% gradient Tris−glycine gels via the Laemmli method.[40] Sample buffer was supplemented with DTT (50 mM) for reducing SDS-PAGE, with about 2 *μ*g of protein per lane. Western blot analysis was performed by blotting onto PVDF, blocking with 1% BSA in buffer W (PBS and 0.1% Tween-20), and incubating with a 1:4000 dilution of His-detector nickel-AP conjugate. We washed the blots in buffer W and developed them with Western Blue stabilized substrate.

LC/FT−MS (liquid chromatography/Fourier transform mass spectrometry) and LC−MS/MS (liquid chromatography

dx.doi.org/10.1021/bi400736c | *Biochemistry* 2013, 52, 5920−5927

tandem mass spectrometry) were performed on tryptic digests as described [34] to determine which Cys residues participated in a disulfide bond. For MALDI−TOF analysis, protein samples were desalted using a C18 ZipTip and spotted with 3,5-dimethoxy-4-hydroxycinnamic acid. We analyzed samples with an UltraFLEX III TOF mass spectrometer in linear mode.

RESULTS AND DISCUSSION

Protein Splicing Activity. We are able to overexpress a fusion of the *Mma* PolII intein with an N-terminal MBP and a C-terminal His tag. The intein was selected for study for two reasons: (i) a noncanonical C-terminal Gln replaces the highly conserved Asn, and (ii) the intein has Cys residues in intein blocks F and G that we suspected might be near the active site and promote disulfide-bond formation (Figure 1B).

On overexpression in *E. coli* and purification utilizing the C-terminal His tag, we observed that the wild-type intein promotes mostly N-terminal cleavage with limited splicing, whereas substitution of the C-terminal Gln165 for the highly conserved Asn results in a considerable increase in splicing efficiency (Figure 2).

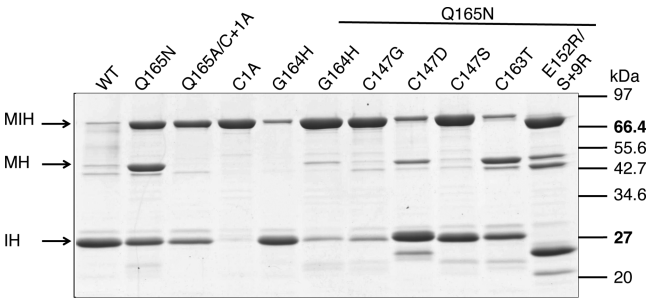


Figure 2. Activity of the *Mma* PolII intein and mutants in *E. coli* BL21(DE3). Reducing SDS-PAGE analysis of proteins after metal-affinity purification. For protein splicing, the precursor MIH (64.8 kDa) is converted to MH (46.0 kDa) and I (18.4 kDa, not detected due to lack of the His tag). For N-terminal cleavage, MIH is converted to IH (20.7 kDa) and M (44.1 kDa). Mutations to MIHMma are given above the lanes; the final six lanes each have the Q165N mutation.

The identity of the bands MIH, MH, and IH were confirmed by Western blot, confirming the presence of a His tag (data not shown), and by MALDI−TOF/MS. For MIHMma, we observed a peak for MALDI−TOF/MS consistent with IH (expected m/z 20 735, observed 20 739). For MIHMma Q165N expressed in *E. coli* BL21(DE3), we observed MALDI−TOF/MS data consistent with IH (m/z expected 20 721, observed 20 712), MH (m/z expected 46 458, observed 46 528), and I (m/z expected 18404, observed 18393). We observed a MALDI−TOF/MS peak consistent with the precursor MIH in the MIHMma Q165N sample expressed from *E. coli* Origami 2(DE3) (m/z expected 64 844, observed 65 026).

Influence of Potentially Catalytic Residues on Splicing Efficiency. It is curious that the intein with native C-terminal Gln splices poorly, given the fact that ligation of the flanking native exteins is likely essential. Cyclization with Gln is slow and results in cleavage of the thioester from steps 1 or 2 uncoupled from splicing, or perhaps the poor splicing in the wild-type context is due to expression in the context of modified exteins and a non-native host organism. Regardless, we show that the control of intein activity by disulfide bond formation described below holds true for both the wild-type intein and a Q165N mutant, so this redox effect is likely physiologically relevant.

In the *Pyrococcus abyssi* PolII (*Pab* PolII) intein, both splicing and C-terminal cleavage via side-chain cyclization are faster with the conserved Asn than with native Gln.[41] The highly conserved penultimate His has been shown to be important in promoting the third step of splicing in some inteins.[42−44] However, in other cases, replacement of nonconserved residues at this position with His has either no effect or is deleterious to splicing.[42,45,46] We hypothesized that replacing the native penultimate Gly residue (Gly164) with the conserved His might increase the extent of splicing with the native Gln. However, substitution of Gly164 for His does not increase the efficiency of splicing in the wild-type *Mma* PolII intein, and it disrupts splicing and N-terminal cleavage in the Q165N mutant (Figure 2), suggesting that the conformational flexibility of the penultimate Gly might be important for coordinating and promoting the steps of splicing.

Besides Cys1 and Cys+1, the *Mma* PolII intein has Cys residues at two important positions. The first is at position four of block F (residue F:4). In other inteins, this residue is the Cys responsible for initiating splicing in class three inteins[47−49] or Asp and Ser residues that may coordinate the steps of splicing.[18,50−53] Although Asp is the most common F:4 residue in canonical inteins, Cys is among the next most conserved options. The second Cys residue is in block G, three residues removed from the active site Cys+1 (Figure 1b). Cys is an unusual residue for this position.[54]

Mutation of conserved Cys residues disrupts splicing (Figure 2). Mutation of Cys1 to Ala, which should prevent step 1, results in precursor accumulation rather than C-terminal cleavage, suggesting that step 1 or 2 of splicing is required to promote step 3. Mutation of both Cys+1 and Q165 to Ala, which blocks steps 2 and 3, results in accumulation of precursor and some N-terminal cleavage, likely from cleavage of the linear thioester formed in step 1.

The MIHMma triple mutant of E152R/Q165N/S+9R was prepared for tryptic digests/LC−MS described below, in order to separate the block F and G Cys residues on different tryptic fragments. The mutation allows for splicing, albeit reduced in extent. We note aberrant migration of the bands IH and I with SDS-PAGE under reducing conditions. However, MALDI−TOF/MS confirmed the identity of these bands as IH (m/z 20 831 observed vs 20 817 expected) and I (m/z 18 443 observed vs 18 431 expected), as does reactivity of IH with the reagent directed against the C-terminal His tag (Supporting Information Figure S1).

Mutation of the block F Cys147 or block G Cys163 also interrupts splicing in MIHMma Q165N (Figure 2). (Although MIHMma promotes mostly N-terminal cleavage, there is a modest effect due to these mutations (Supporting Information Figure S2).) We hypothesized that one of these Cys residues, both likely to be near the active site, might form a disulfide bond that would prevent or regulate splicing. There is a precedent for redox-controlled protein splicing; however, this would be the first example of a native *internal* intein disulfide bond regulating splicing. Although a disulfide bond formed between an internal Cys residue could be inhibitory, it is not surprising that mutation of the Cys residue could interfere with splicing under reducing conditions, as the reduced Cys may play a role in properly coordinating the active site.

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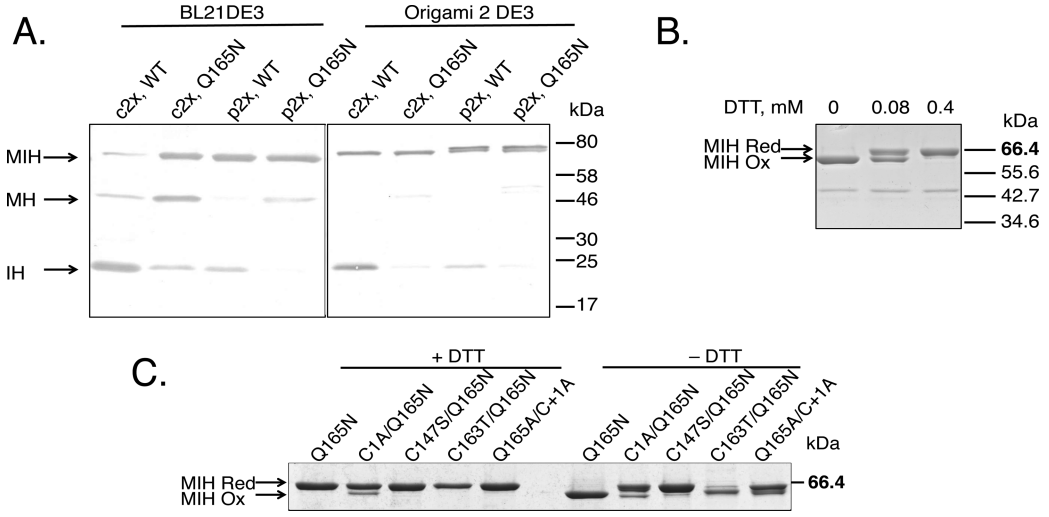

Figure 3. Disulfide bond formation and in vivo splicing or cleavage activity. (A) Western blot analysis of total cellular extracts produced from two separate SDS-PAGE experiments under reducing conditions. Bands indicate the detection of the C-terminal His tag. A lane label of c2x indicates cytoplasmic expression; p2x indicates periplasmic expression. The cell strain is indicated at the top. MIHMma is indicated by WT; MIHMma Q165N is represented by Q165N. (B) Nonreducing SDS-PAGE analysis of MIHMma Q165N purified by metal-affinity chromatography from *E. coli* Origami2(DE3) and incubated with DTT at the given concentrations for 15 min at 50 °C. (C) SDS-PAGE analysis of purified proteins examining the influence of mutations to intein Cys residues on disulfide bond formation, with or without 50 mM DTT in the gel loading buffer.

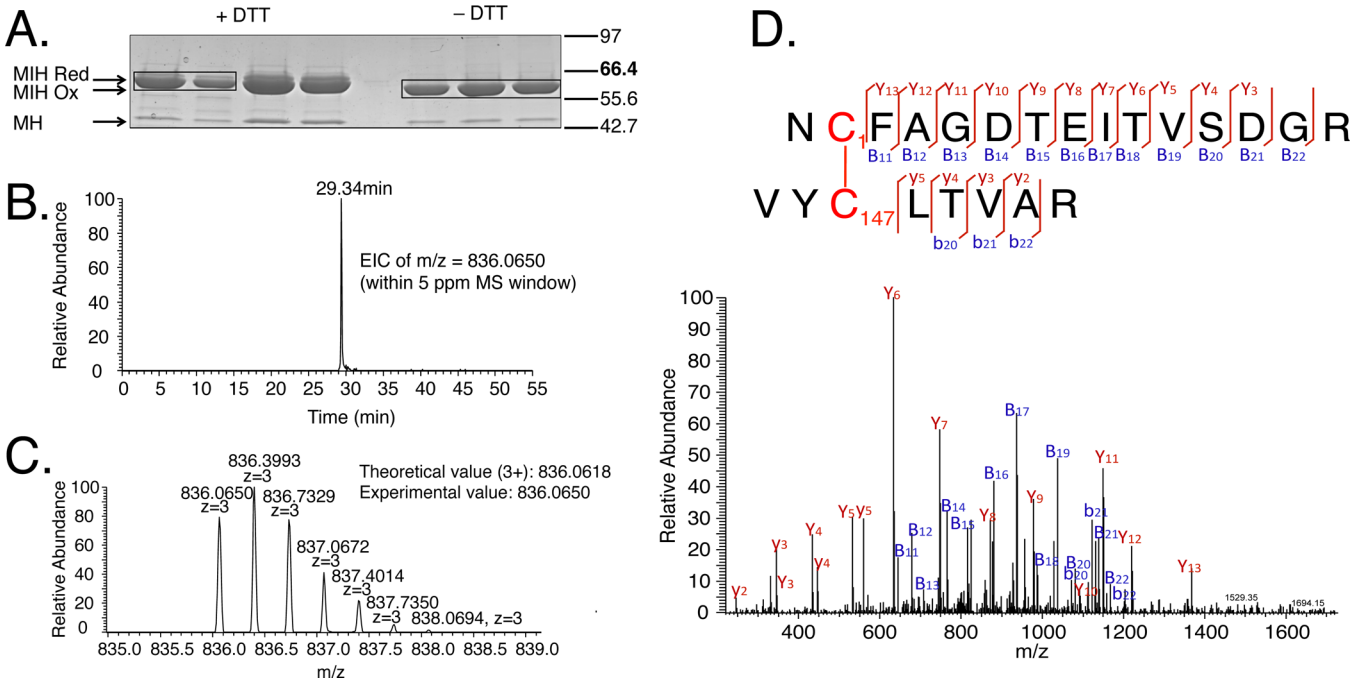

Figure 4. Identification of intramolecular disulfide bond using LC–FT/MS and MS/MS after an in-gel tryptic digest. (A) SDS-PAGE analysis of samples of protein MIHMma Q165N/E152R/S+9R used for tryptic digest and subsequent LC–FT/MS. The first two boxed lanes were used for the "reduced" sample, and the last three boxed lanes were used in subsequent analysis as the "oxidized" sample. (B) Extracted ion chromatography analysis of disulfide peptide (m/z = 836.0650) using accurate MS (within 5 ppm mass accuracy window). The peptide eluted at 29.34 min on a C18 HPLC column in the tryptic digestion of the oxidized sample from SDS-PAGE in A. (C) Experimental FT/MS data with isotope distribution for the identified intramolecular disulfide bond peptide. The mono isotopic mass matched well with theoretical data (m/z = 836.0618, M = 2505.162). (D) MS/MS matching of the intramolecular disulfide bond sequence. Matching data is shown as Table S1 in Supporting Information.

Protein Splicing Controlled by a Disulfide Bond: SDS-PAGE Evidence. For the *Mma* PolII intein, the first evidence of a disulfide bond came from a comparison of protein splicing activities when proteins were overexpressed in *E. coli* BL21(DE3) versus *E. coli* Origami 2(DE3), which has deletions of two oxidoreductases, trxB and gorA, that result in cytoplasmic disulfide bond formation.[55] We noted an increase in unreacted precursor, as well as a decrease in the intensity of bands due to splicing or N-terminal cleavage, when proteins are expressed in the oxidizing *E. coli* Origami 2(DE3) cells rather than the reducing *E. coli* BL21(DE3) cells (Figure 3A).

In addition, we incubated unspliced precursor isolated from *E. coli* Origami 2(DE3) with increasing amounts of DTT. When SDS-PAGE is performed in the absence of additional DTT, the

dx.doi.org/10.1021/bi400736c | *Biochemistry* 2013, 52, 5920–5927

band representing the unspliced precursor has an altered migration that is restored to the expected migration on reduction, suggesting a change in molecular shape because of the presence or absence of a disulfide bond (Figure 3B).

We made separate mutations to each Cys residue in the context of the Q165N mutant to determine which Cys residues might be responsible for making a disulfide bond. We then analyzed the change in migration by SDS-PAGE for the unspliced precursor under reducing or nonreducing conditions (Figure 3C). For Q165N, we observed a change in migration of the precursor band in the absence of reductant, which we attributed to a disulfide bond. However, the shift in migration was lost with the mutation of Cys1 to Ala or Cys147 to Ser, suggesting that these residues were involved in the disulfide bond. We observed a mixed phenotype when Cys+1 is converted to Ala or Cys163 is converted to Thr, suggesting that neither residue is absolutely required for disulfide bond formation (Figure 3C). Mutation of Cys163 to Thr also affects the extent of splicing (Figure 2), so it may play some structural role in the active site. We observe similar gel shifts with wild-type MIHMma (Supporting Information Figure S3).

Protein Splicing Controlled by a Disulfide Bond: Mass Spectrometry Evidence. To verify the Cys residues involved in the disulfide bond, we performed further analysis by mass spectrometry. We purified MIHMma E152R/Q165N/S+9R from *E. coli* Origami 2(DE3) and analyzed the sample by SDS-PAGE both with and without the addition of DTT. The unspliced precursor bands boxed in Figure 4A were excised from the gel and digested by trypsin. We analyzed the digests by LC/FT−MS and MS/MS. We identified an LC peak present in the nonreduced sample, but not present in the reduced sample, with a retention time of 29.34 min (Figure 4B). FT−MS analysis of this fraction gave a $z = 3$ peak of 836.0650, compared to a theoretical value of 836.0618 for a peptide linking the tryptic fragments containing Cys1 and Cys147 (Figure 4C). MS/MS analysis of this fraction was also consistent with this peptide (Figure 4D). We observed over 90% coverage of tryptic peptides for both the reduced and oxidized samples.

In Vitro Activity of Unspliced Precursor. We wanted to determine if we could purify redox-trapped precursor and induce splicing or cleavage in vitro. We purified MIHMma Q165N and incubated the protein at 37 °C at pH 7.5 in the presence of 50 mM DTT, 150 mM DTT, 4 mM TCEP, or no reductant. We observe that DTT induces N-terminal cleavage in a concentration- and time-dependent manner (Figure 5).

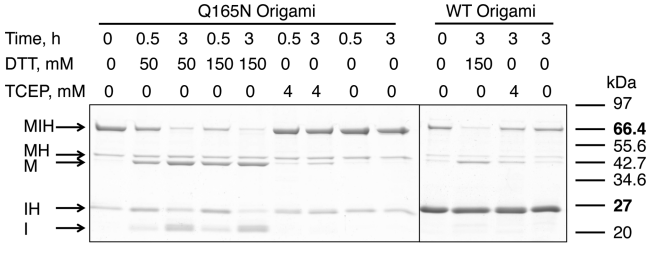

Figure 5. SDS-PAGE analysis of incubation of MIHMma and MIHMma Q165N purified from *E. coli* Origami 2(DE3). In vitro incubation of disulfide-linked precursor with reducing agents resulted in mostly N- and C-terminal cleavage. Proteins were purified as described and exchanged into buffer A supplemented with reducing agents to the final concentration indicated above the lanes, in addition to 4 mM EDTA. Samples were incubated at 25 °C for time indicated.

Incubation with TCEP results in cleavage as well, although less efficiently. This may result from DTT both reducing the disulfide bond and serving as the nucleophile to induce thiolysis of the linear or branched ester. Similar results are seen with the wild-type MIHMma. After N-terminal cleavage, a band appears that migrates consistently with the size of the excised intein. (This band does not react with the anti-His tag antibody, further suggesting that it is I and not IH with aberrant migration. Bands attributed to IH and I were also confirmed with MALDI−TOF/MS.) Although there may be a small amount of splicing generating the I band, it is more likely that the IH formed on N-terminal cleavage and then underwent uncoupled C-terminal cleavage, which is supported by the decrease of the IH band and increase of I band in Figure 5. This suggests that the intein undergoes a mechanism-linked conformational change after step 1 or 2. Therefore, the resulting cleavage of the thioester is required to permit step 3, consistent with precursor accumulation when step 1 of splicing is blocked (Figure 2). Although we isolate less precursor on expression in *E. coli* BL21(DE3) (Figure 3A), treatment of the remaining precursor with DTT or TCEP gives similar results (Supporting Information Figure S4), suggesting that the precursor is catalytically competent to facilitate the steps of splicing, even if they are not properly coordinated. In attempts to induce splicing rather than cleavage, we varied pH, temperature, time of incubation, and the means by which we disrupt the cells. We also added solubilizing reagents and attempted to refold the protein under reducing or nonreducing conditions by both rapid dilution and slow dialysis, and we consistently observed stable precursor with some uncoupled cleavage reactions rather than in vitro splicing (data not shown). However, our in vivo results show that the intein splices under reducing conditions and does not splice under nonreducing conditions, suggesting a potential physiological relevance (see below). Because we are able to induce N-terminal cleavage efficiently in vitro from an inactive precursor, the intein might provide a use in biotechnology applications such as intein-mediated protein purification.

Potential Regulatory Role for an Intein Disulfide Bond. We also wanted to determine if this disulfide bond could control protein splicing in different cell compartments. Either MIHMma or MIHMma Q165N was expressed in the cytoplasm or periplasm. Total cellular extracts were analyzed for splicing activity by reducing SDS-PAGE followed by Western blot (Figure 3A). In the BL21(DE3) strain, splicing and N-terminal cleavage are significantly reduced on expression in the periplasm, which is a more oxidizing environment than the cytoplasm. Similar reductions in activity are observed in Origami 2(DE3) cells. This suggests that this redox trigger allows splicing to be sensitive to the different redox states in different cellular compartments. Given that the native *Mma* host is an obligate anaerobe that can colonize diverse anaerobic environments,[56] it is exciting to speculate that intein-mediated redox control of the activity of the replicative polymerase might have physiological relevance.

Relative Strengths of Intein Disulfide Bonds. It appears that the disulfide bond between residues 1 and 147 was strong enough to be fully oxidized in *E. coli* Origami 2(DE3), particularly when expressed to the periplasm. When expressed in BL21(DE3), the fraction of the precursor that was not oxidized appears to either splice or undergo cleavage. This led us to question whether the disulfide bond in the *Mma* PolII

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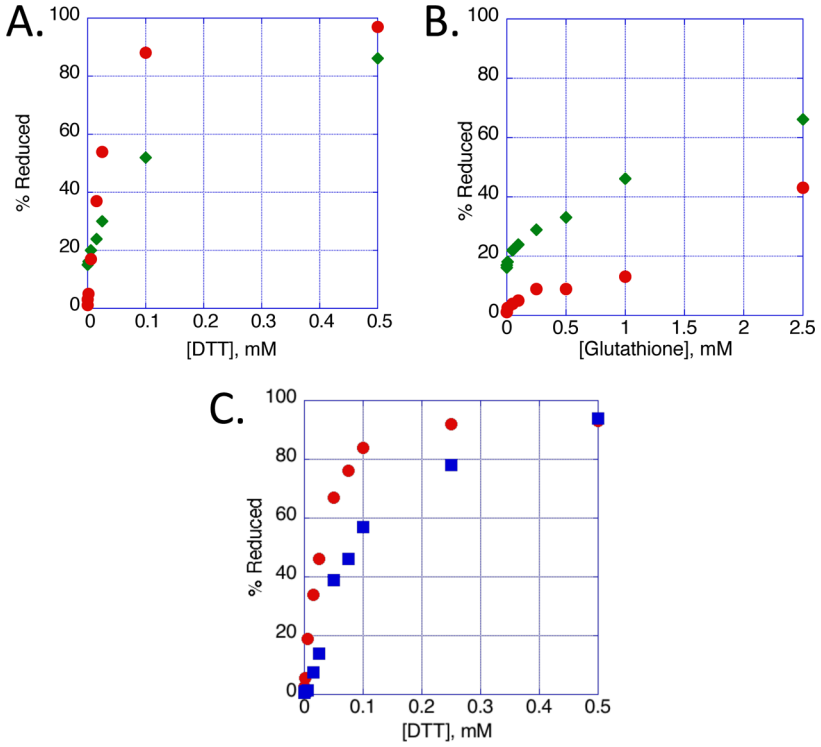

Figure 6. Comparison of reduction of disulfide-linked precursors. We calculated the percent reduction of the unspliced precursor by estimating the relative amount of protein in the oxidized or reduced precursor band by densitometry using ImageJ,[61] such that % reduction = 100 × (Reduced)/[Reduced + Oxidized]. In each case, reduction was for 30 min at 20 °C. (A) Reduction with DTT of *Pab* (◆) versus *Mma* (●) precursor. (B) Reduction with reduced glutathione of *Pab* (◆) versus *Mma* (●) precursor. (C) Reduction of *Mma* precursor by DTT with (●) or without (■) 1 mM EDTA.

intein is more resistant to reduction than the disulfide we previously observed in the *Pab* PolII intein.[34]

We previously reported that the *Pab* PolII intein could be isolated as a disulfide-inactivated precursor on overexpression in *E. coli* BL21(DE3).[34] We also showed that the disulfide bond was sensitive to temperature and flanking sequence. To compare these results with those of the *Mma* PolII intein, we examined the *Pab* PolII intein in the same MIH context, as previously described.[11] We purified both MIHMma and MIHPab from overexpression in *E. coli* Origami 2(DE3). In Figure 6A, we show that although over 10% of our purified MIHPab precursor was in the reduced state, the MIHMma precursor was more easily reduced by DTT on incubation at 20 °C for 30 min with 1 mM EDTA. This could be due to the relative strength of the disulfide bonds and/or to the rigidity of the thermophilic *Pab* intein at low temperature. However, the *Pab* intein was more sensitive to reduction by glutathione under the same conditions (Figure 6B), suggesting that the disulfide bond between Cys1 and Cys+1 of the *Pab* intein might be more attributable to the bulkier reducing agent than the internal Cys1−Cys147 disulfide in the *Mma* intein. The *Mma* intein was also more sensitive to DTT-induced reduction in the presence of 1 mM EDTA (Figure 6C), which we did not observe with *Pab* intein. This might indicate metal ion involvement with disulfide bond formation or in the active site. Divalent cations have been shown to inhibit protein splicing with other inteins.[57−60]

■ ASSOCIATED CONTENT

Supporting Information

MS/MS matching data from Figure 4D; SDS-PAGE and Western blot analyses of proteins. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This material is based upon work supported by the National Science Foundation under grants MCB-0950245 and MCB-1244089 (K.V.M.), by the National Institutes of Health grants GM38060 (R.J.L.) and GM81408 (C.W.), and by a Henry Dreyfus Teacher−Scholar Award (K.V.M.).

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS AND TEXTUAL FOOTNOTES:

MBP, *E. coli* maltose binding protein; MIH, a fusion protein of MBP, an intein, and a poly His tag; MIHMma, MIH with the *Mma* PolII intein; MIHPab, MIH with the *Pab* PolII intein; *Mma*, *Methanoculleus marisnigri*; *Pab*, *Pyrococcus abyssi*

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