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MINIREVIEW

The Role of the Hsp100/Clp Family of Proteins in Prokaryotic Development

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Cells are able to cleanse themselves of unwanted proteins by degrading them and recycling their breakdown products. Targets for degradation can be damaged proteins resulting from encounters with a harsh environment or a toxic agent. They can be proteins that block regulatory pathways that must be activated under specific conditions. Incomplete translation products are also targets of intracellular degradative systems. Components of these same systems monitor protein conformation and remodel proteins and protein complexes to either inhibit or stimulate their activity. These tasks are accomplished by the cell's arsenal of degradative enzymes that are accompanied by their protein-remodeling partners, the molecular chaperones. Molecular chaperones that function in the controlled destruction and remodeling of proteins and protein complexes serve several purposes within the normal operation of cellular processes. They are induced to remove inactive, incomplete, and denatured translation products. They function in rescuing proteins that become denatured or in releasing active monomers from inactive protein aggregates. The elevated activity of proteolytic enzymes that catalyze the disposal of damaged proteins and the induction of chaperones that restore damaged proteins to native activity are both hallmarks of the heat shock response. But these systems are induced in response to other kinds of stress such as extremes of pH and osmolarity as well as encounters with powerful oxidants and membrane-damaging agents. Both membrane and cytosolic proteins are subject to conformation monitoring and proteolytic disposal mediated by protein complexes containing molecular chaperones. These systems also catalyze essential reactions within the cell's signal transduction networks governing responses to changing environmental conditions. Environmental stimuli trigger programmed regulatory switches involving the proteolytic elimination of factors that inhibit signal passage to the proteins mediating the cell's response.

These are processes common to all organisms and are carried out by ubiquitous proteins that exhibit extensive primary structure conservation. Among these is a class of proteins that were originally identified as products of genes that are induced by heat shock, hence referred to as heat shock proteins or HSPs. These include subunits of multi-component proteases that constitute the HSP100/Clp family. Members of this large protein family bind to, denature, and deliver proteins to the catalytic core of proteolytic complexes. They form homo-hexameric or -heptameric complexes in the presence of ATP and utilize the energy released from ATP hydrolysis to catalyze protein unfolding. Thus, the ClpXP and ClpAP complexes (19, 21, 96) implicated in several proteolytic reactions within regulatory pathways, consist of the HSP100 homologs ClpX or ClpA, that present denatured substrate protein to the proteolytic component, ClpP. The ClpXP and ClpAP proteases cleave the substrate polypeptides to yield peptide fragments of 10 amino acids or less. ClpX, as well as other members of the HSP100 family, can catalyze the denaturation of substrate proteins, thereby exposing the polypeptide chain to proteolysis. Their capacity to remodel protein conformation is evident in other processes in which HSP100 proteins play essential roles as molecular chaperones.

Clp structure and activity

The Clp proteins belong to a large family of regulatory ATPases called the AAA family of proteins (ATPases associated with diverse cellular activities) (15, 63) which includes proteins of the 19S regulatory subcomplex of the 26S proteosome of eukaryotes as well as proteins that function in protein trafficing and organelle biogenesis. All possess one or two copies of a 180~200 amino acid sequence that encompasses the nucleotide-binding domain of the protein. Alignments of amino acid sequences reveal distinct classes of proteins within the Hsp100/Clp family of AAA proteins. Class 1 members, including HSP104,

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ClpA, ClpB, ClpE, and ClpC, contain two ATP-binding domains and conserved, signature amino acid sequence motifs within three regions (29, 73) (Fig. 1). The N-terminus (region I), the region between the two ATP-binding domains (regions II and III), and the C-terminus (regions IV and V) bear distinct amino acid sequence motifs that distinguish the separate Class 1 proteins. ClpE of *Bacilus. subtilis* (10) and *Lactococcus lactis* (29) possesses a Zinc-finger motif within region I, distinguishing it from ClpC (7, 29, 58, 72), the other prominent Class 1 Hsp100/Clp family member of Gram positive bacteria. The Class 2 members, which in Gram positive bacteria are represented by HslU(ClpY) (53) and ClpX (20), possess only one ATP-binding domain, but share amino acid sequence motifs within regions IV and V found in members of Class 1 (29). But, like ClpE, ClpX possesses a Zinc-finger at its extreme N-terminus (Fig. 1A). The function of the Zinc-finger motif of ClpE and ClpX is not known, but is thought to participate in the protein's interaction with denatured polypeptides, as might be the case in the co-chaperone, DnaJ (81). Interestingly, the *Saccharomyces cerevisiae* and *Neurospora crassa* homologues of ClpX, Mcx1 (87), (NCBI access.AL355933.1), do not possess Zn-finger motifs. This might be a reflection of the fact that the *S. cerevisiae* genome does not encode a product homologous to ClpP, suggesting that the Zn-finger may mediate interaction between ClpX and ClpP. The conserved C-terminal region encompassing motif V has been termed the "sensor-substrate discriminator domain" (SSD) (79) and is found in all regulatory ATPases of the Hsp100/Clp family, including the single polypeptide ATP-dependent proteases Lon and FtsH. The SSD of bacterial ATP-dependent proteases can be purified as polypeptides of between 12 to 16 kD that recognize substrate proteins, but show overlapping specificity (79). Thus, ClpA and ClpX SSD domains bind to proteins tagged with the *ssrA*-encoded amino acid sequence that is normally appended to incomplete translation products, a modification that marks truncated proteins for proteolytic elimination.

It is not known if the presence of two nucleotide-binding domains (NTP) in the Class 1 HSP100/Clp members reflects a function or reaction mechanism that distinguishes Class 1 from Class 2 proteins. Recent studies of Wickner, Maurizi and colleagues show that ClpA, a Class 1 member, is able to bind to partially denatured GFP, while ClpX requires a specific recognition tag, like SsrA, in order to bind GFP (28, 76). The presence of the additional nucleotide binding domain might augment the binding capacity of the ATPase and/or the affinity to denatured polypeptides.

There is very little structural information for many of the Clp proteins, with the most thorough analysis coming from the characterization of HslU(ClpY) (4) (Fig. 2A). Crystals of hexameric HslU, with and without AMP-PNP, were obtained as well as crystals of HslUV(ClpYQ) protease complex. Both N- and C-terminal domains of HslU interact with ATP and non-hydrolyzable analogs, an interaction that results in movement of the two domains towards one another. Several residues of the N-domain, containing the consensus Walker A and B motifs (Fig. 1A) contact the bound ATP as does the highly conserved arginine of the signature sequence V of the C domain. The intermediate (I) region, that bisects the N-terminal domain, protrudes inward to partially occupy the lumen created by the ring-like HslU hexameric structure. The I domain also protrudes downward to form the interface with the proteolytic subunit HslV (Fig. 2A). While this model of HslU complex architecture and subunit disposition might be relevant for HslUV protease structure, it probably does not apply to the structure of ClpXP. As shown in the alignment of Fig. 1B, ClpX possesses an N-terminal nucleotide binding region and a C-terminal domain that are homol-

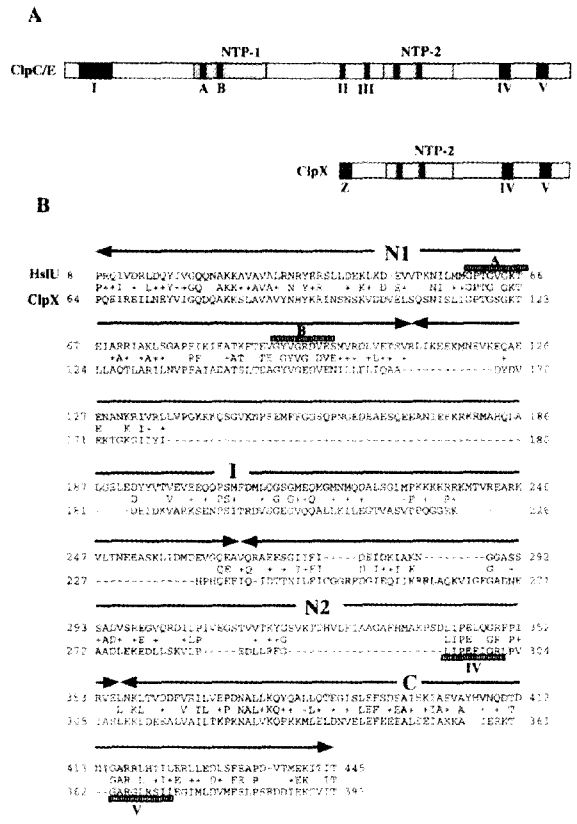

Fig. 1. **(A)** A schematic diagram of Clp primary structure. Shown are the locations of the signature sequences of Class 1 (shown here as ClpC/E) and Class II (ClpX) ATPases. The locations of nucleotide binding domains 1 (NTP-1) and 2 (NTP-2) are shown along with the sites of the conserved Walker A and B motifs. The sites of the signature sequences are shown as I, II, III, IV, and V. Z marks the location of the Zn-finger motif of ClpX. **(B)** Alignment of *B. subtilis* HslU (ClpY) and ClpX amino acid sequences. The locations of the N, I, and C domains are indicated and are based on the alignment of the B. subtilis and *E. coli* HslU sequences as well as the crystal structure analysis of HslU (4). Shown are the locations of the Walker A and B motifs (A and B with striped over-bar) and signature sequences IV and V (with striped under-bar).

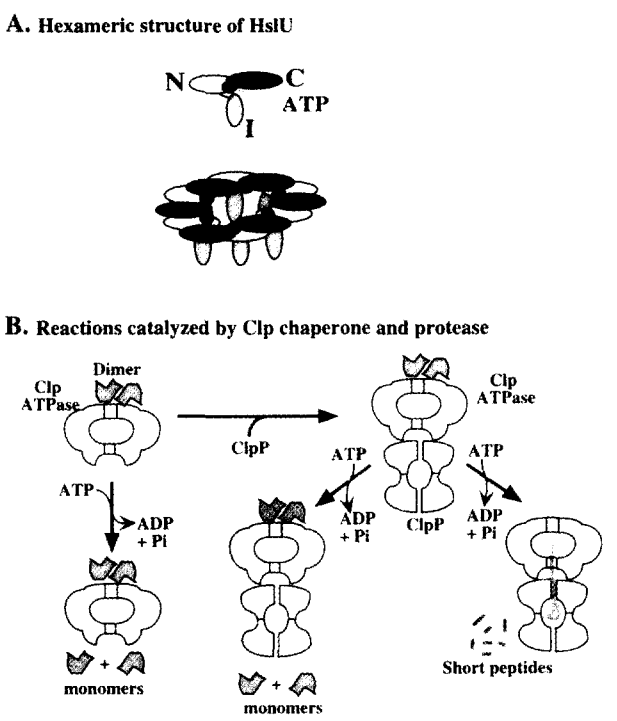

Fig 2. (**A**) diagram of the monomeric and hexameric structure of the HslU ATPase based on the crystal structure analysis of Bochtler et al. (4). N, I, and C represent the N-terminal, intermediate, and C-terminal domains. Approximate location of the bound ATP is shown. (**B**) A schematic diagram of the reactions catalyzed by Clp ATPase and Clp protease complexes. Structures are loosely based on the electron micrographic observations of Clp protein complexes (33). The Clp substrate is depicted as a dimer which undergoes dissociation to the monomeric form by the action of either Clp ATPase or the ATPase complexed with ClpP. Although the diagram shows the association of Clp ATPase at one end of the ClpP complex, a Clp ATPase hexamer can also bind to the other end of the ClpP axial pore.

ogous to those of HslU, but little primary or secondary structure similarity with the I and adjacent, downstream N-domain region (N2, Fig. 1B) can be detected in the ClpX amino acid sequence. The I domain contains the sites of contact with the proteolytic, HslV subunit, which shows no sequence similarity with the proteolytic component of ClpXP, the ClpP protein. The lack of sequence similarity within the internal sequences of ClpX and HslU may be a reflection of the differences in ATPase and proteolytic core interaction between the two proteases.

The structures of Clp ATPases and ClpP complexes have undergone analysis involving analytical ultracentrifugation, cryo-electron microscopy, and X-ray crystallography (23, 33, 51, 90,91). The C- and N-terminal regions of Clp ATPases are the sites of multimerization, as has been observed with other multimeric AAA protein complexes (74) (Fig. 2A). This interaction is facilitated by the binding of ATP or its non-hydrolyzable analogs. In this form, the Clp ATPase can catalyze protein unfolding, a process requiring ATP hydrolysis (77, 92).

The hexamers of ClpA or ClpX interact with heptameric rings of ClpP (3, 23, 51, 83) (Fig. 2B). The ClpP rings are assembled back-to-back so that the axial pores at each end of the ClpP complex can interact with a Clp ATPase hexamer. It is this unusual assymetric coupling of ClpA/X with ClpP that has generated interest among those attempting to relate Clp protease structure to function (3). Recent reports included the proposal that interaction between the ClpAT-Pase and ClpP multimers is mediated through single subunit pairs. Interaction rapidly shifts to an adjacent pair, resulting in rotation of the ATPase and proteolytic complexes relative to one another (3). If the unfolded polypeptide substrate is being passed from ATPase through the axial pore of the ClpP heptamer, then this rotational movement might further stimulate mechanical denaturation of substrate that is initiated within the Clp ATPase complex (3, 34). Thus, the assymetric pairing of the ATPase and ClpP complexes is thought to facilitate unfolding by mechanical means rather than through a strictly chemical mechanism. Indeed, ClpP was shown to enhance the unfolding activity of Clp ATPases and to suppress mutations conferring defects in Clp activity (31, 34, 66).

Recent studies, utilizing two novel reagents, have provided a detailed picture of Clp-catalyzed protein unfolding (depicted in Fig. 2B). Three groups (28, 34, 92) have used the artificial Clp substrate GFP-SsrA, composed of the green fluorescent protein, or an unstable mutant derivative, and the SsrA degradation tag normally encoded by the *ssrA* RNA of *E. coli* (32). The latter is appended to the C-terminus of GFP. Denaturation of the substrate can be monitored by measuring the loss of fluorescence brought about by interaction of GFP-SsrA with a Clp ATPase. The other reagent is a mutant form of GroEL called GroEL-(92), which irreversibly binds to denatured protein. The interaction of GroEL-Trap with denatured protein formed as a result of Clp-catalyzed polypeptide unfolding provides another measure of Clp activity. The studies showed that ClpX and ClpA can bind substrate proteins in the presence of ATP and catalyze the denaturation of protein, a process requiring ATP hydrolysis. When bound to ClpP, proteolysis proceeds through 5 steps (34) : 1) protein binding to the Clp ATPase component; 2) denaturation of the protein; 3) translocation of the polypeptide substrate to ClpP; 4) degradation of the substrate; 5) release of peptide products. Because the rate of denaturation equals the rate of degradation, step 2 is thought to be the rate-limiting step in the reaction cycle. Step 5, product release is accelerated by binding of a second substrate polypeptide and is dependent on ATPase activity. This can result in the release of intact or incompletely degraded polypeptide. This could be the result of ClpP axial pore opening which is mediated by Clp ATPase, the activity of which is stimulated by the binding of substrate. These studies showed that remodeling of protein conformation can be accomplished by the ATPase complexed with the proteolytic component, but is also cat-

alyzed by Clp ATPase.

Regulatory role of Clp proteases in developmental processes:* Caulobacter *cell cycle, control of RpoS concentration, and genetic competence

The stalked bacterium, *Caulobacter crescentus* undergoes a process of cellular differentiation that is coupled to the cell cycle [reviewed in (95)]. Like eukaryotes, and unlike many bacteria, the *C. crescentus* cell cycle proceeds through distinct stages (G_1, S, and G_2/M) and is accompanied by a single round of chromosome replication involving one replication initiation and one termination event (Fig. 3A). A protein of the response regulatory class of the two-component family of proteins, CtrA, prevents the initiation of DNA replication in the swarmer cell by

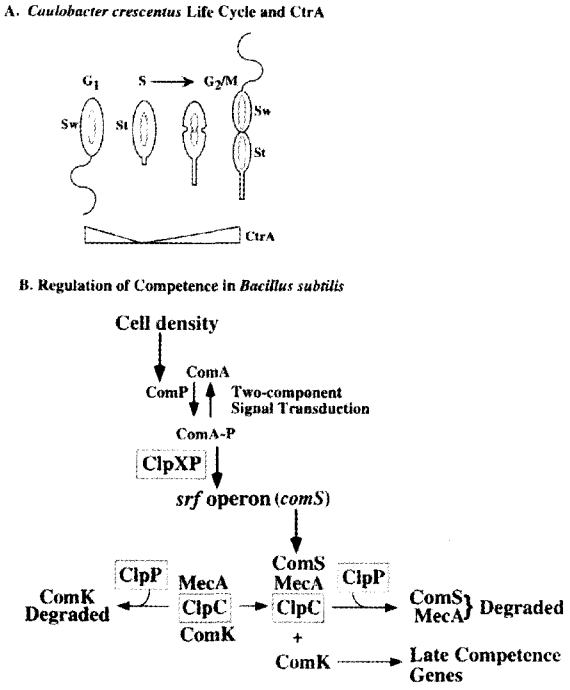

Fig. 3. **(A)** A diagram of the developmental cycle of *Caulobacter crescentus* showing the replicaiton incompetent swarmer cell (Sw) bearing the single polar flagellum and at the G_1 phase of the cell cycle. The stalk cell stage is depicted as St which is also the stage at which DNA synthesis begins (the S phase). Division of the stalk cell to generate a swarmer cell and the parent stalk cell is shown at stage G_2/M. The oscillation of CtrA concentration is depicted below the cell cycle illustration. **(B)** A diagram of the regulatory pathway of *B. subtilis* competence establishment is shown. Signals derived from high cell density are channeled into the ComPA two-component signal transduction system. Autophosphorylated ComP donates a high energy phosphate to ComA which, now in its active form, activates the transcription of the *srf* operon. The subsequent production of ComS peptide results in the release of ComK from the MecA/ClpC complex. Released ComK is now free to activate the transcription of the late competence genes as well as its own gene. ComS also directs the degradation of MecA by ClpCP. In the absence of ComS, ComK is degraded by ClpCP, a process that is mediated by the recognition factor MecA.

preventing the expression of genes required for DNA synthesis and cell division (68, 97). To initiate DNA replication in the stalk cell compartment, CtrA must be eliminated proteolytically. The oscillation of CtrA concentration parallels the process of chromosomal replication through the developmental cycle. Null mutations in either *clpP* or *clpX* were observed to be lethal, as was a mutation that conferred a substitution at the active site serine residue of ClpP (30). The phenotype of *clpX* and *clpP* prompted the examination of the relationship between CtrA concentration and the essential function of ClpXP. By controlling the expression of *clpP* or *clpX* genes fused to a xylose-inducible promoter, Jenal and Fuchs showed that depletion of either ClpX or ClpP results in an increase in CtrA concentration and cell cycle arrest at G_1, or before the beginning of S phase when DNA synthesis is initiated (30). The activity and/or concentration of ClpXP are thought to be under tight cell cycle control in *C. crescentus*. Indeed, the utilization of one of the three promoters that drives *clpX* transcription is negatively influenced in the swarmer cell, which could contribute to the elevated concentration of CtrA.

Another regulatory target of ClpXP protease is the RNA polymerase sigma subunit, RpoS, or σ^s of Gram negative organisms that is active when conditions that create stress are encountered (27). Activation of RpoS and the increase in its concentration result in the transcription of 50 to 100 genes that confer general resistance to stress and toxic agents (82). The expression, concentration and activity of RpoS are under complex control involving transcriptional regulators (cAMP-CRP and BarA) (38, 60), post-transcriptional control mediated by an RNA binding protein (Hfq) (59) and regulatory RNAs [DsrA (41, 48) and OxyS (98)], and proteolytic regulation that is catalyzed by ClpXP (99), but is mediated by a phosphoprotein, RssB (MviA) (2, 55, 67). RpoS is an unstable protein during periods of robust growth, with a half-life of 2 mins. But the turnover rate is reduced 10-fold in response to a shift to high osmolarity, temperature, or low pH. Under these conditions, the RssB protein is down-regulated through an unknown mechanism. RssB is a member of the response regulator class of the two-component regulatory family of proteins. It possesses the characteristic and conserved N-terminal or receiver domain, but contains a C-terminal domain unlike any known protein. Studies involving *in vitro* binding assays and *in vivo* two hybrid analysis showed that phosphorylated RssB interacted with RpoS (2, 55), contacting a domain of the sigma subunit that is necessary for instability (the D-box, or destruction box). Upon binding, RssB presents RpoS, normally not a substrate of ClpX, to the ClpXP complex for proteolysis. Stress conditions are thought to promote the dephosphorylation of RssB, thereby rendering RpoS resistant to ClpXP-catalyzed destruction. Thus RssB acts as a recognition factor that promotes controlled proteolytic turnover.

Another example of controlled, Clp-catalyzed proteolysis involving a recognition factor is found in the developmental pathway governing the establishment of competence development in *B. subtilis* (11, 12, 84). The establishment of genetic competence is characterized by the emergence of a subpopulation in a culture grown in glucose-minimal medium, that becomes endowed with the ability to take up exogenous DNA. Competence is induced by high cell-density and starvation conditions through the activity of extracellular peptide factors (39, 40, 47) (Fig. 3B). These accumulate to high concentration and activate a two-component system composed of the membrane-bound histidine protein kinase, ComP (94) and the response regulator ComA (70). Phosphorylated ComA stimulates the transcription of the *comS* gene (9, 26), encoding a small peptide that is required for the expression of competence genes, the products of which directly function in the uptake of exogenous DNA. These genes require the transcription factor, ComK (89), which is normally unstable under conditions of low cell density that do not promote competence establishment. Mutations that obviate the need for *comS* in the activation of competence gene expression reside in the *mecA* and *clpC* genes (13, 25, 71). MecA is a protein that interacts with ComK (35, 36), thereby inhibiting its activity, but it requires the ClpC protein for efficient interaction (86). The ternary complex of MecA, ClpC, and ComK sequesters the competence transcription factor (86), but also targets it for proteolytic destruction by ClpCP (85). The *comS* gene product, by interacting with MecA (65, 86), promotes the release of ComK, thereby preventing its destruction and rendering it active to stimulate competence gene transcription. Like RssB, MecA serves as a recognition factor that presents a substrate, ComK, to the ClpCP protease. Instead of dephosphorylation, MecA is inhibited by an intracellular regulatory peptide, ComS, that is controlled by a quorum-sensing device composed of the ComPA signal transduction system.

ClpXP also plays a role in the SOS response in *E. coli* (16), both in the adaptive turnover of proteins whose production is induced by DNA damage and in restriction alleviation, which protects newly synthesized DNA from restriction enzyme cleavage. UmuDD' heterodimers are degraded by ClpXP (16) as part of the adaptation process involving the downregulation of PolV concentration. PolV ($UmuD'_2C$) (64) catalyzes trans-lesion DNA synthesis required to negotiate damage areas of the genomic DNA during replication. Type I restriction enzymes are also targets of ClpXP when DNA is synthesized in response to DNA damage (49). The newly synthesized DNA is sensitive to the Type I, multi-component restrictionmodification enzyme complex. ClpXP eliminates the HsdR component that catalyzes DNA cleavage. Genetic transfer of the type I restriction modification system from one strain to another also requires ClpXP to prevent HsdR-catalyzed cleavage of recipient genomic DNA before modification of sensitive sites can be performed by the HsdM methylase (50). ClpXP targets only the form of HsdR that is able to catalyze ATP-dependent DNA strand-translocation, suggesting that HsdRM that is associated with the DNA target is the specific substrate of ClpXP. This substrate selectivity of ClpXP, targeting only proteins that are bound to DNA, is observed in other systems (Mu transposition, see below) and may indicate that the ClpXP system is designed not only to recognize the state of the substrate protein, but also the state of the nucleic acid bound to the substrate. In this case, only the unmodified DNA can induce the Type I restriction enzyme down the path of restriction cleavage, the state of the enzyme that is targeted by ClpXP.

Clp proteins as chaperones in the regulation of processes involving protein-DNA interaction: Phage development and transcription initiation.

Phages are adept at exploiting the cellular processes of the sensitive host cell. The lytic phage Mu, which amplifies its genome in infected cells by replicative transposition, utilizes a molecular chaperone of the host cell to control the initiation of DNA synthesis so that it begins after strand transfer (31, 42, 52). The MuA protein catalyzes strand transfer in which a MuA tetramer pairs the ends of the phage genome DNA and cleaves them. Then, with the aid of MuB (1), joins the ends of the phage DNA with a target DNA site, forming the transpososome (54). After strand transfer, MuA remains bound and in this form can block replication of the phage genomic DNA. The MuA complex then undergoes a conformational change that renders its association with the phage-target DNA junction heparin sensitive. This remodeling of MuA requires the host ClpX protein, with optimal activity requiring the ClpXP complex (37, 42). This interaction does not involve proteolysis of MuA, which is eventually released after DNA synthesis commences. ClpX-dependent remodeling of MuA only occurs after strand transfer, because the MuB protein binds the site of MuA that is recognized by ClpX (43). A 10 amino acid C-terminal sequence of MuA is recognized by ClpX, and can be transferred to heterologous proteins, thereby converting them to ClpX substrates (34, 79). How the ClpX protein remodels the MuA-DNA complex to allow replication of the phage DNA is not known.

While ClpX plays important roles in the developmental cycles or lytic and temperate phages of *E. coli*, deletion of the *clpX* and *clpP* genes of *E. coli* does not significantly affect bacterial growth (75), although there is a loss of resistance to heat and DNA damaging agents. In some bacteria, *clpP* and *clpX* play more important roles and are essential in some bacterial species. As described above, null mutations of *clpP* and *clpX* are lethal in *C. crescentus*. The ClpX and ClpP proteins, while not essential,

are crucial factors in the complex response of *B. subtilis* to nutritional stress and high cell density (18). As a culture of *B. subtilis* enters stationary phase, cells choose from various possible adaptive responses depending on growth conditions (24). They will produce extracellular degradative enzymes and antibiotics. Motility will be induced temporarily, but is down-regulated at high cell density (46). Two processes of cellular differentiation will be induced. One is genetic competence that was described above, and the other is sporulation, a process that produces the highly resistant cell type called the endospore (80). Both *clpX* and *clpP* are necessary for cells to initiate the sporulation process (18, 44, 56, 57). They can exert their influence on the signal transduction processes governing sporulation gene transcription. One of these processes is the Spo0 phosphorelay (5) that is responsible for the accumulation of phosphorylated Spo0A, the key transcriptional activator of sporulation genes. Signals originating from environmental and metabolic conditions feed into the phosphorelay via histidine protein kinases KinA and KinB, which auto-phosphorylate and donate phosphate to the response regulator Spo0F. The protein phosphotransferase Spo0B then transfers the phosphate from Spo0F to Spo0A, converting Spo0A to its active form. In addition to the phosphorelay, the alternative RNAP sigma subunit, σ^H is necessary for the transcription of some sporulation genes as well as the optimal transcription of the *spo0A* gene (6, 14, 78). The σ^H protein is encoded by the *spo0H* gene, and, as the name implies, is the site of mutations that prevent the initiation of sporulation, or blocking the sporulation process at stage 0. A null mutation of *clpX* prevents the post-exponential phase activation of σ^H-dependent transcription (44, 45), but has little direct effect on the concentration of σ^H or the amount of σ^H co-purifying with RNA polymerase (45). We propose that the chaperone activity of ClpX is responsible since a *clpP* mutation did not affect expression of genes that bear promoters which are recognized by the σ^H-form of RNA polymerase (44). The ClpX protein, when added to a reaction containing a σ^H-specific linear DNA template and purified RNA polymerase (45), stimulates transcription initiation as evidenced by the accumulation of run-off transcript. Single-cycle reactions, in which a second round of initiation is prevented by the addition of heparin to the reaction, appeared not to be affected by the addition of ClpX, suggesting that ClpX functions at a step of transcription after the binding of RNA polymerase to the promoter DNA. One could imagine that ClpX might catalyze a macromolecular dissociation resulting in the removal of σ^H and thereby allowing RNA polymerase to enter the elongation stage of transcription. This would be akin to the function of ClpX in promoting Mu DNA replication by dissociating MuA from the strand-transfer complex. Alternatively, ClpX could prevent the aggregation of RNAP or σ^H, thus maintaining the proteins in an active form.

ClpX also is required for another kind of regulated transcription initiation in *B. subtilis*. This was discovered in the study of *srf* operon transcription. The *srf* operon encodes the peptide synthesizing enzyme complex, surfactin synthetase (8, 17, 88) and also contains the gene that codes for the competence regulatory peptide ComS (9, 26). As described above, the transcription of *comS*, and the entire *srf* operon, requires the two-component regulatory system consisting of ComP and ComA (9, 26, 62, 70, 93, 94). Phosphorylated ComA accumulates as a result of ComX phermone-dependent stimulation of ComP kinase activity (47). ComA phosphate binds to the "ComA boxes", two regions of dyad symmetry, residing upstream of the *srf* operon promoter (70). In doing so, it is thought to recruit RNAP to the *srf* promoter to initiate transcription. *In vivo*, ComA-dependent stimulation of *srf* operon transcription is dependent on both *clpX* and *clpP* (61). The requirement for *clpX* and *clpP* is not alleviated by a mutant allele of *comP* that renders the ComP histidine kinase constitutively active (61), suggesting that the activity of ClpX and P are exerted at the level of ComA phosphate activity or *srf* transcription initiation.

The isolation of suppressor mutations in the *rpoA* gene that bypass the requirement for *clpX* with respect to both *srf* and σ^H-dependent activity (61) suggested that ClpX exerts its effect on the transcription initiation complex. The two suppressor mutations were found to reside in the region of *rpoA* encoding the C-terminal domain of the RNA polymerase alpha subunit. The αCTD is known to function both in RNAP-promoter interaction (22) and in the contact between RNAP and positive regulators of transcription (69). These suppressors did not overcome the requirement for ClpX in conferring normal levels of heat resistance as *clpX* strains bearing either of the suppressors were still heat sensitive. The suppressors might allow RNA polymerase to proceed into the elongation phase of transcription without the aid of ClpX. We do not know why there is an additional requirement for ClpP, but one could imagine that the ClpXP-dependent proteolytic removal of ComA-Phosphate might be necessary for the conversion of the transcription initiation complex to the elongating form of RNAP. While it is premature to propose a specific model for how ClpX and ClpP affect regulated transcription in *B. subtilis*, the results suggest that ClpX functions intimately with the transcription initiation complex to stimulate transcription.

Future prospects

Using the *E. coli* genetic system and detailed structural analysis, a wealth of information has been gained that has brought into sharp focus the nature and activity of the Hsp100/Clp family of molecular chaperones. What is still lacking is a detailed description of Clp chaperone activity, specifically the mechanisms by which the Clp ATPases

are able to recognize protein substrates and then remodel protein conformation. Recent studies using other well-developed genetic systems have uncovered unique associations that place the Clp proteins in the most fundamental of cellular processes such as chromosome replication and transcription initiation. This view has been reinforced by the extreme pleiotropy of *clp* mutations and by the results of experiments involving the reconstruction of Clp protein activity in transcription reactions assembled *in vitro*. The essential nature of Clp proteins, particularly ClpX and ClpP, in macromolecular synthesis might be a more common observation as the genetics and biochemistry of ClpX and ClpP from other bacterial species are examined.

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