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| No. | Reference | Summary | Notes |
| 1 | Rocaboy,M. et al. (2013) The crystal structure of the cell division amidase AmiC reveals the fold of the AMIN domain, a new peptidoglycan binding domain. Molecular Microbiology, 90, 267–277. | Describes the crystal structure of the AmiC NAMLA amidase in *E. coli*, the only protein structure for this amidase in *E. coli* currently published. | This unbound AmiC protein structure in *E. coli* is the only crystal structure currently described for the amidase in *E. coli*. Resolving the structure of AmiC identified two distinct domains: the catalytic Amidase domain and the N-terminal AMIN domain. The amidase is made from 6 alpha-beta structures, which is highly conserved between amidase\_3 family proteins, and is potentially auto-inhibited by an alpha helix obstructing the active site when cofactor NIpD is not bound.  The AMIN domain is less well established, and theorised to bind to and stabilise peptidoglycan due to a repeated recognition sequence which is predicted to bind to peptidoglycan. Also has two specifically conserved regions, where the second region may stabilise a folding intermediate form, or bind to a different molecule involved in the septal peptidoglycan cleavage process. |
| 2 | Peters,N.T. et al. (2011) A Fail-Safe Mechanism in the Septal Ring Assembly Pathway Generated by the Sequential Recruitment of Cell Separation Amidases and Their Activators. Journal of Bacteriology, 193, 4973–4983. | Uses protein localisation assays to identify order of protein interactions between AmiA, AmiB, AmiC and their LytM factors. | Until this paper, the order of protein binding in the splitting of the septal ring during bacterial cell division wasn’t established. By looking at which proteins could localise independently to the septal ring and which depended on other proteins being localised already, a relationship between the proteins involved can be established.  NIpD activates AmiC, however they both require FtsN to already be localised to the septal ring before they localise. AmiB also requires FtsN to be localised, whereas its LytM activator EnvC doesn’t need FtsN to localise. And when the peptidoglycan is not synthesised (blocked by penicillin), then the LytM factors localise, but none of the amidases do. Therefore: FtsN localises first, then the LytM factors can localise with or without peptidoglycan, but both FtsN and peptidoglycan are needed before the amidases can localise, which may be a ‘failsafe’ to ensure the PG ring is synthesised before it is hydrolysed by the amidases. |
| 3 | Yang,D.C. et al. (2012) A conformational switch controls cell wall-remodelling enzymes required for bacterial cell division. Molecular Microbiology, 85, 768–781. | Describes structure of an amidase in *Bartonella henselae* and proposes existence of self-regulating helix in gram negative autolytic amidases. | Describes the crystal structure of an amidase (called as and ‘AmiB ortholog’ in the paper) in a gram negative bacteria, with a focus specifically on comparison to other amidase (AmiB, AmiC) sequences as were available at publication. So in addition to the crystal structure, the paper performs a multiple sequence alignment against several other gram negative NAMLA amdase orthologs, as well as NAMLA domain amidases in 4 bacteriophages and in 3 amidases proposed to be involved in cell lysis of parent cell in sporulating bacteria.  The alignment was on very few sequences, but shows that all the gram negative orthologs had an around 50bp insertion, which the crystal structure predicted to be an alpha helix obscuring the active site of the *B. henselae* amidase. Used this to propose three types of amidase: endolytic (for bacteriophages), cell lysis (for sporulating bacteria), and a specific cell separation amidase unique to gram negative bacteria, which might be identifiable from the other two types by this alpha helix region. |
| 4 | Cong,Q. et al. (2019) Protein interaction networks revealed by proteome coevolution. Science, 365, 185–189. | Uses protein-protein interaction model to identify coevolution between *E. coli* protein pairs. | Using the residue-residue mutual information statistical model as a measure for each residue pair, the model screens out which pairs of the 4262 *E. coli* proteins are most strongly predicted to co-evolve. The paper then used *E. coli* complexes from the PDB as a positive control set, and top 961,929 pairs selected for GREMLIN, and then final selection using model docking with distance constraints to select 804 protein pairs. (this is not improved by deep learning!)  Coevolution varied across the dataset, with stronger coevolution in binary interfaces and weaker with nucleic acid binding. Methods used to identify orthologs for *E.coli* and using GREMLIN to analyse coevolution valuable for this project, as well as one protein identified as strongly coevolving with AmiC (bepA – Beta-barrel assembly-enhancing protease) |
| 5 | Chodisetti,P.K. et al. (2022) A LytM-Domain Factor, ActS, Functions in Two Distinctive Peptidoglycan Hydrolytic Pathways in E. coli. Front Microbiol, 13, 913949. | ActS identified as part of the septal ring splitting pathway (and one other pathway) as an activator of AmiC – potentially binds to AMIN domain? | There are four main LytM proteins; MepM (endopeptidase activity, cell wall elongation), EnvC and NIpD (septal ring division), and ActS. For division, FtsZ recruits other proteins, eventually leading to EnvC and NIpD activation of amidases, however this paper describes the activity of ActS in the complex, showing through protein expression analyses (eg western blotting) that ActS suppresses MepS (involved in elongation) and activates AmiC while localising to the septal ring, but is not itself a hydrolase and may activate a separate pathway which hydrolyses peptidoglycan in place of MepS.  It could be possible that this is the protein that the AmiC AMIN domain also binds to, identified in the Rocaboy et al. paper from 2013? Study suggests that the asp-149 residue on ActS is essential/highly conserved and might coordinate with the zinc ion in AmiC to activate it (which is within the active site behind the auto-inhibitory alpha helix – ActS shown to localise later, so perhaps this is alongside displacement of the helix by NIpD?) |
| 6 | Boelter,G. et al. (2022) The lipoprotein DolP affects cell separation in Escherichia coli, but not as an upstream regulator of NlpD. Microbiology, 168, 001197. | DolP identified as involved with cell wall separation, perhaps interacting with AmiC - potentially binds to AMIN domain? | DolP (also known as YraP) is an outer membrane protein which is shown in this paper through peptidoglycan degradation assays and various knockdown studies to not interact with, regulate, or affect AmiC activity with NlpD, but the AmiC-NlpD complex needs DolP to function correctly, and there is also evidence DolP may interact weakly with AmiC (and AmiA).  DolP has a BON domain and localises to the division site, so is very likely to be involved. Another candidate for the mystery AMIN domain interactor? Paper suggests the interaction may be transient between AmiC and DolP, and help AmiC to be recruited to the divisome. |
| 7 | Garcia,D.L. and Dillard,J.P. (2006) AmiC functions as an N-acetylmuramyl-l-alanine amidase necessary for cell separation and can promote autolysis in Neisseria gonorrhoeae. J Bacteriol, 188, 7211–7221. | Survival and autolysis assays of NAMLAA in *N. gonorrhoeae*, show function of NAMLAA in both cell separation and cell lysis (when present in excess) | Examined role and action of AmiC (*E.coli* homologuein *N. gonorrhoeae*, another gram negative bacteria). Found two NAMLAA in the *N. gonorrhoeae*, however one was a homologue for AmpD (cytoplasmic amidase).  When inserting a deletion within the amidase gene, cell separation was shown to be deficient in mutant cells (looking at the cells using transmission electron microscopy). Also, the study found that autolysis still occurred even when amiC was mutated (in fact an increased rate of cell lysis), suggesting that other proteins are involved in autolysis (and that in gram negative bacteria, the function of NAMLAA is more in cell wall separation rather than autolysis)    (above: From Garcia and Dillard, 2006 – A: Wild type amiC, C: mutated amiC (cell separation deficient, unlike as typically seen for wild type amiC) |
| 8 | Feliciano,C.A. et al. (2021) A lipoprotein allosterically activates the CwlD amidase during Clostridioides difficile spore formation. PLOS Genetics, 17, e1009791. | Crystal structure of a NAMLAA ortholog from a sporulating bacteria, suggests regulation via Zn2+ cofactor (and not self-regulation by helix) | Role of amidases in sporulating bacteria like *C. difficile* stated to be for degrading the peptidoglycan ‘cortex’ to allow budding of new bacterial cell. To examine regulation of these amidases (as could by via self-regulating region like for gram negative or by cofactor binding), crystal structure was solved and examined by this paper. Found that the GerS lipoprotein and Zn2+ may regulate function.  Ligand formation was shown between GerS and Zn2+ and therefore inhibitory effect predicted (and confirmed through several protein function assays with/without Zn2+ and key GerS residues), however the structure does not have a helix within the active site. For the *B. henselae* and *E. coli*, the same ligands are formed with a helix in the active site – providing evidence for the autoinhibitory effect of this additional helix? (helix also notably absent from this sporulating bacteria structure)    (above: from Feliciano *et al.*, 2021 – on the right are the  *B. henselae* and *E. coli* structures, compared to ‘active’ sporulating bacteria structures on the left, which lack the helix) |
| 9 | Korndörfer,I.P. et al. (2006) The Crystal Structure of the Bacteriophage PSA Endolysin Reveals a Unique Fold Responsible for Specific Recognition of Listeria Cell Walls. Journal of Molecular Biology, 364, 678–689. | Structure of a bacteriophage NAMLAA ortholog (substrate for NAMLAA in gram positive bacteria) | Despite being in a bacteriophage, this NAMLAA is highly specific to Listeria cell wall – a gram positive bacteria – and consists of a catalytic domain and a cell wall binding domain (which was shown through a functional assay to be required for full functionality).  The catalytic domain was confirmed to be NAMLA amidase\_3 by aligning with a sporulating bacteria amidase sequence (no sequence from a NAMLA amidase involved with cell wall separation, however did show close alignment with the sporulating amidase, ie having no additional ‘gram negative’ helix – had an RMSD of 1.61 to the sporulating amidase structure too. Could be either because it targets gram positive cell wall, or from being present within a bacteriophage?) |
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| -- | Levin,P.A. and Janakiraman,A. (2021) Localization, Assembly, and Activation of the Escherichia coli cell division machinery. EcoSal Plus, 9, eESP00222021. | [not for final biblio, for reference only] A review of studies which describe the assembly of the divisome in E. coli | 1. FtsZ localises to midcell site first (a GTP hydrolase), associates in a ‘Z-ring’ arrangement. It assembles peptidoglycan cross-wall formation. 2. FtsA and ZipA mediate association of FtsZ to the membrane at the midcell. FtsA undergoes polymerisation 3. In order, several transenvelope structural and peptidoglycan remodelling proteins associate in coordination with cell cycle progression. 4. FtsK: DNA translocase (moves chromosomal DNA away from the septal ring, recruits FtsQLB complex) 5. FtsQ, FtsL + FtsB complex: Assembly of the rest of the divisome. Periplasmic, localised mid-cell, L and B recruit W,I and N. 6. FtsW and FtsI (involved with cross-wall synthesis): W is transmemberane (10-pass!), links N-acetylglucosamine and N-acetylmuramic acid for the peptidoglycan backbone. FtsI is a penicillin-binding protein (PBP3) and catalyses peptide stem links between peptidoglycan subunits. Activation of these shift the complex from elongation to cross-wall formation. 7. FtsN (has a SPOR domain and is the last of these proteins to associate at the divisome). Has a cytoplasmic domain, a periplasmic domain, and a SPOR domain that reacts with glycans in the peptidoglycan which are exposed by hydrolases in remodelling the cell wall during growth/separation. Connects the cytoplasmic, periplasmic, and membrane parts of the complex. 8. Confirmation change occurs in the divisome and cell wall synthesis is stimulated, with FtsZ distributing the other factors/enzymes around the septal ring, making cross-wall links. 9. Cross-wall links constrict the outer membrane, making a ‘dimple’ at either side of the cell (at this point there is a new layer of peptidoglycan running across the cytosol at the mid-cell) 10. The outer layer of the septal peptidoglycan is cleaved by amidases alongside peptidoglycan synthesis. AmiA is localised throughout the periplasm in any cell cycle. AmiC is activated by outer membrane proteins NlpD and DolP 11. The Tol-Pal complex creates the dimple/invagination; TolQ, TolA, TolR at the cytoplasmic membrane, TolB in the periplasm, and Pal in the outer membrane (TolB and Pal form a complex that diffuses through the outer membrane when division is not happening). 12. Membrane fusion within the daughter cells happens, splitting the cytoplasm. 13. The amidases separate the septal peptidoglycan walls 14. The outer membranes are ‘sealed’ 15. The divisome dissembles and the daughter cells separate.   Modulated by FtsEX – FtsX interacts with FtsA to enhance FtsA activity, and FtsE interacts with FtsZ. Also promotes amidase activity through interaction with EnvC.  Paper has also collected a PPI table for the Fts protein complex under standard lab growth conditions. |