

Lysis from without

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In this commentary I consider use of the term “lysis from without” (LO) along with the phenomenon’s biological relevance. LO originally described an early bacterial lysis induced by high-multiplicity virion adsorption and that occurs without phage production (here indicated as LO_v). Notably, this is more than just high phage multiplicities of adsorption leading to bacterial killing. The action on bacteria of exogenously supplied phage lysin, too, has been described as a form of LO (here, LO_L). LO_v has been somewhat worked out mechanistically for T4 phages, has been used to elucidate various phage-associated phenomena including discovery of the phage eclipse, may be relevant to phage ecology, and, with resistance to LO (LO_R), is blocked by certain phage gene products. Speculation as to the impact of LO_v on phage therapy also is fairly common. Since LO_v assays are relatively easily performed and not all phages are able to induce LO_v, a phage’s potential to lyse bacteria without first infecting should be subject to at least in vitro experimental confirmation before the LO_v label is applied. The term “abortive infection” may be used more generally to describe non-productive phage infections that kill bacteria.

for efficient macromolecular synthesis and the cell simply dies.”

Ian Molineux¹ (p. 223)

Ambiguity in terminology hinders scientific progress. Examples of ambiguous terms in phage biology include multiplicity of infection²⁻⁴ and pseudolysogeny,⁵ which have taken on multiple, often difficult to distinguish meanings. Use of the term lysis from without (LO) has similarly drifted from its original meaning.

Lysis from within (LI) is normal bacterial lysis induced intracellularly by phage proteins. LO, by contrast, is a lysis that does not rely on phage infection but instead is effected directly by extracellularly supplied agents.^{6,7} Notwithstanding the simplicity of this definition, for more than half a century many authors have invoked LO imprecisely, emphasizing phage and bacterial *failure to survive* rather than strictly adsorption-induced bacterial lysis. Molineux,¹ by contrast and as quoted above, distinguishes LO from simply phage and bacterial death. Since how LO is defined can have consequences, here I consider its usage. Those consequences include within the context of phage therapy, which is the application of phages to control nuisance or pathogenic bacteria.^{3,8}

Key words: abortive infection, bacteriophage, lysin, lysis, lysis from without, phage therapy

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Introduction

“The phenomenon of lysis-from-without is exhibited by T-even and certain other phages with large genomes, but does not appear to be particularly widespread. Most phages do not cause lysis-from-without; more often, high multiplicities of infection simply overwhelm all capacity

Virion-Mediated LO (LO_v)

The most-cited early LO_v reference is that of Delbrück,⁹ who seems to have coined the term, though earlier descriptions of LO_v-like phenomena also exist.¹⁰ Delbrück treated cultures containing 10⁸ *Escherichia coli*/ml with sufficient phage numbers that a measured “saturation”

in adsorption was achieved. Earlier lysis occurred than when lower phage numbers were supplied, around 10 min versus after 15 min, reflecting LO versus LI, respectively. Lack of production of phage progeny was also observed.

LO_v as mediated by phage ghosts can serve in the laboratory as a simple and relatively rapid means of lysing bacteria.¹¹ Consistently, the phage eclipse was discovered, in part, via LO_v of phage infections during single-step growth.^{10,12-14} For more modern examples of LO_v experiments, see Asami et al.¹⁵ For images of bacterial cells post LO_v, see Cota-Robles and Coffman¹⁶ and Tarahovsky et al.¹⁷ In addition, I have identified over 300 references that mention or discuss LO. These I present as **Supplementary Material**, distinguished in tabular form into various categories as discussed here, including in terms of what papers consider LO_v within the context of phage therapy.

Molecular Mechanisms

LO_v has been most studied in T-even phages, particularly phages T2 and T4.^{6,7,18} It occurs as a consequence of phage penetration through the bacterial cell envelope during adsorption. This penetration is effected in T4 phages by the gp5 protein, which is a tail-associated lysozyme.¹⁸ When few phages adsorb to individual bacteria, then the damage caused by gp5 is relatively slight and does not lead to premature bacterial lysis. However, when substantial numbers of phages adsorb, then sufficient cell-wall damage can occur that lysis follows. This lysis may occur principally at “weak points” in the bacterial envelope¹⁷ and that envelope, under at least some circumstances, may not be “extensively degraded.”¹⁶

In T-even phages there exists a complication on the above mechanism, termed resistance to lysis from without (here, LO_R). LO_R can be observed when there is a delay between primary phage adsorption and high-multiplicity secondary phage adsorptions.¹⁹ LO_R is dependent on phage gene expression and, in T4 phages, is associated especially with expression of the phage gene *sp* plus to a lesser extent the phage gene *imm*.⁶ The existence of LO_R can result in false-negatives in LO_v assays,

meaning that phages which otherwise can exhibit LO_v might not display LO_v even if high multiplicity adsorption occurs. This failure is particularly likely if high multiplicity adsorption occurs after the first few minutes of phage infection. Alternatively, display of LO_R by infections can be blocked by inhibiting gene expression.

Existence of the LO_R mechanism^{6,7} helped to illuminate the mechanics of normal phage T4 adsorption.¹⁸ Lysis inhibition,⁶ as required for high-titer T4 stock production, also seems to involve expression of LO_R.^{20,21} The lysis of T4 cultures, as during stock preparation, can involve a LO_v-like lysis mechanism,²² though one that is not necessarily independent of LI.²³ Alternatively, Mg²⁺ addition to media (25 mM) can reduce at least *E. coli* susceptibility to LO_v.²⁴ Phage λ produces proteins, Rz and RZ1, that appear to be important for effecting LI also given higher divalent cation densities such as LB broth supplemented with 10 mM MgCl₂.^{25,26} Cell envelopes under the low Mg²⁺ conditions typically observed during in vitro phage characterization thus may be generally less resistant to lysis than under other circumstances. Fresh cells, contrasting those that have been subject to refrigeration, also have been found to display a lower susceptibility to LO_v.²⁴ Together these various mechanisms and observations are suggestive that while LO_v could have ecological as well as applied relevance, at the same time LO_v may be less readily induced than one might anticipate. In particular, interfering mechanisms can include gene expression during phage infections such that LO_R is induced, insufficiently rapid or extensive phage adsorption (as discussed below), or given various conditions that can result in what may be greater cell-envelope resistance to this lysis.

Abortive Infection as it Relates to LO_v

LO_v, as a bacterial killing mechanism in which adsorbing phages also are sacrificed, can be viewed as a kind of abortive infection.¹⁴ Abortive infections, however, can occur due to a great many causes besides LO_v.^{27,28} Phage infections that result in both phage and bacterial death therefore should not be assumed to be due

to LO_v, even given substantial multiplicities of phage adsorption, without some kind of substantiation that extracellularly induced early lysis has occurred, or at least could occur in the case of speculation as to in situ behavior such as during phage therapy.

Formation of clearings upon application of high-titer droplets of phages to the surface of immature bacterial lawns (spot testing) similarly is not necessarily an indication of LO_v since such spots—strictly, zones of inhibition—can be formed due to other abortive infection mechanisms or due to crude-lysate-associated lysins or bacteriocins. Indeed, phage-induced bacterial death associated with high multiplicities of phage adsorption can result simply from normal phage infection and associated LI. See **Table 1** for consideration of how to parsimoniously distinguish between productive infections, abortive infections, and lysis from without based upon various hypothetical experimental observations.

Assaying for LO_v

At a minimum, LO_v is a bacterial lysis that occurs soon after phage application. Microscopic or turbidimetric measurements, or detection of the liberation of relatively large intracellular contents such as β-galactosidase are used to detect this lysis. This is rather than determinations of bacterial viability since phage adsorption and infection, alone, typically will kill bacteria. Basic testing for the occurrence of LO_v should be performed if during experiments an unexpectedly low phage productivity from phage-adsorbed bacteria is observed or if one is concerned about negative consequences of high levels of phage adsorption such as during phage therapy.

In designing LO_v assays it is important to keep in mind that while the total number of phages that can adsorb a bacterium is a function of the ratio of phages added to bacteria along with the bacterium's phage-adsorption *capacity*,^{10,14} the actual rate that bacteria become phage adsorbed is a function of phage density.²⁻⁴ High titer phage stocks (≥10⁹ phages/ml) thus should be used in combination with substantial excesses of phages over bacteria

Table 1. Causes of bacterial death associated with high MOI_{actual} parsimonious interpretations

$MOI_{actual}^{1,2}$	Bacterial death ³	Lysis timing ⁴	Phage release ⁵	Default interpretations ⁶
Higher	With lysis	Early	No	LO_v at higher MOI_{actual} ; productive infection at lower MOI_{actual}
Lower	With lysis	Normal	Yes	
Higher	With lysis	Early	No	LO_v at higher MOI_{actual} ; non-productive phage infection at lower MOI_{actual}
Lower	Yes or no	Normal or never	No	
Higher	With lysis	Early	No	Abortive infection ⁷ (potentially also LO_v at higher MOI_{actual})
Lower	With lysis	Early	No	
Higher	CFU loss	no data	No	High MOI_{actual} -dependent abortive infection ⁸ (LO_v ?)
Lower	CFU loss	no data	Yes	
Higher	CFU loss	no data	no data	Productive infection (but perhaps abortive infection)
Lower	CFU loss	no data	no data	
Higher	CFU loss	no data	Yes	Productive infection
Lower	CFU loss	no data	Yes	
Higher	Spot formation	no data	no data	Abortive infection (potentially also LO_v at higher MOI_{actual}) ⁹
Lower	No plaques	no data	no data	
Higher	Spot formation	no data	no data	Productive infection (possibly also footnotes 8 and 9 or LO_v at higher MOI_{actual})
Lower	Plaques	no data	Yes (implied)	

¹"MOI," Multiplicity of Infection; " MOI_{actual} " is the number of phages that have been found to have *adsorbed* to bacteria, within a culture, divided by the number of bacteria present within the same culture, as can be approximated through a combination of measuring phage titers prior to exposure to bacteria (P_0), phage titers after phage exposure to bacteria (but before phages have productively lysed bacteria; P_t), and bacterial counts as present prior to phage addition to bacteria, B_0 , such that $MOI_{actual} = (P_0 - P_t)/B_0$ (contrast MOI_{input} which is equal to the number of phages that have been added to a culture divided by the number of bacteria that are found within the same culture, or P_0/B_0). ²"Higher (MOI)," $MOI_{actual} \gg 1$; "Lower (MOI)," approximately $MOI_{actual} \leq 1$; "no data," specific assays either were not initiated or were not successfully completed. ³"With lysis," bacterial death associated with phage-induced bacterial lysis; "CFU," bacterial-Colony-Forming Unit (losses are declines in CFU numbers associated with a culture); "Spot," clearing initiated by substantial phage numbers suspended within a small volume that's dropped onto an immature bacterial lawn and which results from an inhibition of bacterial replication as typically mediated via bacterial killing; "Plaque," visible clearing on a bacterial lawn that is initiated by approximately a single phage, or single phage-infected bacterium, and which is a consequence of multiple rounds of productive phage infection. ⁴"Early," lysis that occurs substantially prior to the normal time of lysis; "Normal," lysis timing of ordinary productive phage infections as determined especially in the course of single-step growth experiments. ⁵"Phage release" is as following productive infection (and is implied given plaque formation). ⁶"Lysis from without (LO_v)," phage-induced bacterial lysis that is directly associated with phage adsorption rather than with factors that are synthesized within the subsequently lysing bacterium; "Productive infection," phage infection that ends with release of newly produced free phages into the extracellular environment; "Abortive infection," phage infection that ends with both phage and bacterial death; "Non-productive phage infection," infection that does not release phage progeny (could be restrictive, abortive or lysogenic). ⁷Certain abortive infections involve bacterial lysis, especially early lysis, that that is not necessarily associated with LO_v . ⁸Abortive infection sensu Molineux;¹ see opening quote of article; LO_v must be ruled out (or in) via further experimentation. ⁹Also bacteriocinogeny, which is the result of bacteriocins contaminating the applied phage stock during spot testing, or carryover of otherwise intracellularly acting lysins, both as may be found within crude phage lysates.

(>100 phages per bacterium) to effect LO_v . These details are less of a concern if LO_v is readily experimentally induced but should be the first issues considered if LO_v is not observed. As LO_v represents not just lysis but premature lysis, the rapidity of that lysis should be compared with that of normal LI unless LO_v occurs so quickly (<5 min) that it is unlikely to be due to LI.

Note that greater temperatures can increase both the rate and degree of LO_v experienced by bacteria.¹⁷ Since LO_v should not be dependent on phage-lysate-contaminating lysins or bacteriocins,

it should still occur even given virion purification. Also, since LO_v should not be dependent on post-adsorption bacterial or phage gene expression, it should still occur even given washing of target bacteria with nutrient-free buffer prior to phage application. The latter in fact will typically make bacteria more rather than less susceptible to LO_v , at least so long as phage adsorption can still occur. Importantly, not all attempts to demonstrate LO_v in specific phages have been successful (see " LO_v not observed," **Supplemental Materials**).

Lysin-Mediated LO (LO_L) and Other Variations

Lysins are phage-derived, cell-wall-degrading lytic enzymes that can be used to lyse especially Gram-positive bacteria "from without."²⁹ This LO_L is equivalent to LO_v in that it is induced by an exogenously supplied agent (here lysin), results in bacterial lysis, and is not associated with phage production. Though in my opinion the lytic action of exogenously supplied lysin on bacteria is a legitimate use of "lysis from without," in exploring the

literature it is important to not confuse the two concepts, LO_v versus LO_L . Also confusing, the aquatic phage literature refers to streptomycin-induced bacterial lysis as a lysis from without (LO_s).³⁰ Additional, non-phage uses of LO also exist though these are not explored here.

Conclusion

Cell destruction that is directly mediated by virion adsorption has been recognized as a distinct form of phage-induced lysis at least since 1940. That paper by Delbrück⁹ is available online, including through PubMed, and is as relevant today as it was then in defining the phenomenon. In its summary (p. 660) he notes: "Lysis from without is caused by adsorption of phage above a threshold value. The cell contents are liberated by a distension and destruction of the cell wall. The adsorbed phage is not retrieved upon lysis. No new phage is formed." Notwithstanding this description, LO_v is not always easily induced upon phage adsorption unless phage densities are high, cells are inhibited in their gene expression, or cell envelope stability otherwise is low. In addition, not all phages may be inherently capable of inducing lysis from without. If there is reason to suspect that LO_v might be interfering with phage therapy or other experiments then at least in vitro testing should be performed.

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Note

Supplementary materials can be found at: www.landesbioscience.com/journals/bacteriophage/article/13980

References

- Molineux IJ. Fifty-three years since Hershey and Chase; much ado about pressure but which pressure is it? *Virology* 2006; 344:221-9; PMID: 16364752; DOI:10.1016/j.virol.2005.09.014.
- Kasman LM, Kasman A, Westwater C, Dolan J, Schmidt MG, Norris JS. Overcoming the phage replication threshold: a mathematical model with implications for phage therapy. *J Virol* 2002; 76:5557-64; PMID: 11991984; DOI: 10.1128/JVI.76.11.5557-5564.2002.
- Abedon ST, Thomas-Abedon C. Phage therapy pharmacology. *Curr Pharm Biotechnol* 2010; 11:28-47; PMID: 20214606.
- Abedon ST. Bacteriophages and Biofilms: Ecology, Phage Therapy, Plaques. New York, NY: Nova Science Publishers 2011.
- Abedon ST. Disambiguating bacteriophage pseudolysogeny: an historical analysis of lysogeny, pseudolysogeny, and the phage carrier state. In: Adams HT, Ed. *Contemporary Trends in Bacteriophage Research*. New York, NY: Nova Science Publishers 2009; 285-307.
- Abedon ST. Lysis and the interaction between free phages and infected cells. In: Karam JD, Ed. *The Molecular Biology of Bacteriophage T4*. Washington, DC: ASM Press 1994; 397-405.
- Young R. Bacteriophage lysis: mechanisms and regulation. *Microbiol Rev* 1992; 56:430-81; PMID: 1406491.
- Abedon ST. Kinetics of phage-mediated biocontrol of bacteria. *Foodborne Pathog Dis* 2009; 6:807-15; PMID: 19459758; DOI: 10.1089/fpd.2008.0242.
- Delbrück M. The growth of bacteriophage and lysis of the host. *J Gen Physiol* 1940; 23:643-60; PMID: 19873180.
- Stent GS. *Molecular Biology of Bacterial Viruses*. San Francisco, CA: WH Freeman and Co. 1963.
- Ou CT, Matsumoto I, Rozhin J, Tehen TT. Enzyme assay in cultures of *Escherichia coli* by a continuous flow method based on lysis from without by a phage ghost. *Anal Biochem* 1978; 88:357-66; PMID: 100023; DOI: 10.1016/0003-2697(78)90433-5.
- Doermann AH. Intracellular growth of bacteriophage. *Year Book Carnegie Inst Wash* 1948; 47:176-82.
- Doermann AH. The intracellular growth of bacteriophages I. liberation of intracellular bacteriophage T4 by premature lysis with another phage or with cyanide. *J Gen Physiol* 1952; 35:645-56; PMID: 14898042.
- Adams MH. *Bacteriophages*. New York, NY: Interscience 1959.
- Asami K, Xing XH, Tanji Y, Unno H. Synchronized disruption of *Escherichia coli* cells by T4 phage infection. *J Ferment Bioeng* 1997; 83:511-6; DOI: 10.1016/S0922-338X(97)81129-4.
- Cota-Robles EH, Coffman MD. Electron microscopy of lysis from without of *Escherichia coli* B by coliphage T2. *J Ultrastruct Res* 1964; 10:305-16; PMID: 14166295.
- Tarahovsky YS, Ivanitsky GR, Khusainov AA. Lysis of *Escherichia coli* cells induced by bacteriophage T4. *FEMS Microbiol Lett* 1994; 122:195-9; PMID: 7958773.
- Arisaka F, Kanamaru S, Leiman P, Rossmann MG. The tail lysozyme complex of bacteriophage T4. *Int J Biochem Cell Biol* 2003; 35:16-21; PMID: 12467643; DOI: 10.1016/S1357-2725(02)00098-5.
- Visconti N. Resistance to lysis from without in bacteria infected with T2 bacteriophage. *J Bacteriol* 1953; 66:247-53; PMID: 13096470.
- Abedon ST. Bacteriophage T4 resistance to lysis-inhibition collapse. *Genet Res* 1999; 74:1-11; PMID: 10505404.
- Buller CS, Dobbs K. T4-coliphage infection of *Escherichia coli* with defective cell envelopes. *Biochem Biophys Res Com* 1971; 43:658-65; PMID: 4998188; DOI: 10.1016/0006-291X(71)90665-6.
- Abedon ST. Lysis of lysis-inhibited bacteriophage T4-infected cells. *J Bacteriol* 1992; 174:8073-80; PMID: 1459956.
- Couse NL. Control of lysis of T4-infected *Escherichia coli*. *J Virol* 1968; 2:198-207; PMID: 4911852.
- Puck TT, Lee HH. Mechanism of cell wall penetration by viruses II. demonstration of cyclic permeability change accompanying virus infection of *Escherichia coli* B cells. *J Exp Med* 1955; 101:151-75; PMID: 13233443.
- Berry J, Savva C, Holzenburg A, Young R. The lambda spanin components Rz and Rz1 undergo tertiary and quaternary rearrangements upon complex formation. *Protein Sci* 2010; 19:1967-77; PMID: 20734329; DOI: 10.1002/pro.485.
- Summer EJ, Berry J, Tran TA, Niu L, Struck DK, Young R. Rz/Rz1 lysis gene equivalents in phages of Gram-negative hosts. *J Mol Biol* 2007; 373:1098-112; PMID: 17900620; DOI: 10.1016/j.jmb.2007.08.045.
- Hyman P, Abedon ST. Bacteriophage host range and bacterial resistance. *Adv Appl Microbiol* 2010; 70:217-48; PMID: 20359459; DOI: 10.1016/S0065-2164(10)70007-1.
- Labrie SJ, Samson JE, Moineau S. Bacteriophage resistance mechanisms. *Nat Rev Microbiol* 2010; 8:317-27; PMID: 20348932; DOI: 10.1038/nrmicro2315.
- Loessner MJ. Bacteriophage endolysins-current state of research and applications. *Curr Opin Microbiol* 2005; 8:480-7; PMID: 15979390; DOI: 10.1016/j.mib.2005.06.002.
- Weinbauer MG. Ecology of prokaryotic viruses. *FEMS Microbiol Rev* 2004; 28:127-81; PMID: 15109783; DOI: 10.1016/j.femsre.2003.08.001.