sponding serine-accepting sRNA would permit it to pair exactly with the amber codon UAG. However, that species of sRNA capable of recognizing the UCG serine codon would now be eliminated unless there existed more than one cistron for the UCG-specific sRNA. Furthermore, this model does not explain the release of a large proportion of polypeptide fragments in the Su+ cell, unless we include the additional hypothesis that an amber-specific chain-terminating sRNA exists.

A second precise-reading model is based on the assumption that an sRNA species exists that normally recognizes the amber codon as chain-terminating. A modification of this sRNA could enable it to accept serine. The production of a mixture of complete and incomplete protein would be a consequence of this model if the charging of the altered amber sRNA with serine were inefficient—that is, if not all such sRNA molecules became charged.

In the case of the ambiguous-reading model, no assumption about the nature of chain termination is necessary. Suppression could be caused by a modification of a serine-accepting sRNA, which destroys the specificity of its attachment to a codon without altering its anticodon. For example, modification of the overall structure of the serine-accepting sRNA species which recognizes the serine codon UCG might permit this sRNA species to recognize either the nonsense (UAG) or the serine (UCG) codon. This model requires no additional hypotheses to explain the production of a mixture of fragments and completed chains in the Su+ cell.

The amber suppressor mutation, in the second and third models, could be a mutation either in the sRNA cistron or in a gene coding for an enzyme which could modify the serine-accepting or amber-specific sRNA species assumed to act as the suppressor. Likely candidates for such a task are enzymes which produce the unusual bases in sRNA (for example, methylated bases, pseudouracil, or dihydrouracil). Our results do not allow us to distinguish between enzymatic and genetic modification of the specific sRNA involved. It is possible, therefore, that the new serine-accepting sRNA is only the indirect product of the *su-A* suppressor gene.

Finally, the conditions selected for the mixing experiment (Fig. 6) enable us to determine whether the suppressor mutation is dominant in vitro. Since, in these experiments, the incubation mixtures contained a twofold excess of Su− serine-accepting sRNA over Su+ serine-accepting sRNA, we conclude that the *su-A* suppressor is dominant. This is consistent with the findings of Signer, Beckwith, and Brenner (*25*) that a cell heterozygous for amber suppressor gene is permissive.

MARIO R. CAPECCHI
GARY N. GUSSIN
Biological Laboratories, Harvard University, Cambridge, Massachusetts

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8. Abbreviations and definitions: RNA, ribonucleic acid; mRNA, messenger RNA; sRNA, soluble RNA; ATP, adenosine 5′-triphosphate; GTP, guanosine 5′-triphosphate; pfu, plaque forming units; S-30, a bacterial homogenate from which whole cells, cell-wall fragments, and cell-membrane fragments have been removed by centrifugation at 30,000*g* for 30 minutes; "preincubation," refers to incubation of the S-30 in the absence of labeled amino acids to eliminate amino acid incorporation directed by endogenous *E. coli* messenger RNA; "charged sRNA," is sRNA to which an amino acid is attached by a phosphate-ester linkage between the terminal adenylic acid of sRNA and the carboxyl group of the amino acid; "stripped sRNA," is sRNA from which the amino acid has been removed by hydrolysis of the phosphate-ester linkage; UAG and UCG are symbols representing the sequence of nucleotides in specific codons, the letters represent the nucleotides uridine, adenosine, guanosine, and cytidine 5′-monophosphate; TCA, trichloroacetic acid.
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Pasteurella pestis: Role of Pesticin I and Iron in Experimental Plague

Abstract. *Loss of the genetic determinant for pesticin I in* Pasteurella pestis *results in concomitant loss of the plague coagulase and fibrinolytic factor. The median lethal dose for mice of an isolate lacking only these activities is increased by factors of about 10^1, 10^4, and 10^7 cells when administered by the intravenous, intraperitoneal, and subcutaneous routes, respectively. Virulence of the aforesaid strain can be enhanced in mice treated with 40 μg of ferrous iron. This response resembles that of* Pasteurella pseudotuberculosis, *a closely related species that normally lacks pesticin I.*

Burrows and co-workers (*1*) have described four properties that are essential for full virulence in *Pasteurella pestis*, the causative agent of bubonic plague. These properties are the genetic potentials that permit synthesis of purines (*Pu*+), virulence antigens (*VW*+), and capsular antigen (*F1*+) and permit formation of pigmented colonies on synthetic medium (*P*+). The median lethal doses in mice and guinea pigs of mutants lacking each of these determinants are shown in Table 1. Production of F1 antigen is not essential for virulence in the mouse, and strains that are *Pu*+, *VW*+, and *P*− can be restored to full virulence in this animal by injection of ferrous ion (*2*).

Wild-type strains of *P. pestis* produce at least two bacteriocin-like substances. The first, termed pesticin I (PI), prevents growth of certain strains of *Pasteurella pseudotuberculosis* (*3*, *4*)

and PI-deficient mutants of *P. pestis* that remain P^+. Pesticin II is a second bacteriocin-like substance produced by both *P. pestis* and *P. pseudotuberculosis*; it is active against many strains of *P. pestis* that lack pesticin I (*4*).

We have shown that an absolute correlation exists between production of pesticin I and expression of the plague coagulase (C) and fibrinolytic factor (F). This finding suggests, but does not prove, that *PI*, *C*, and *F* reside on a hereditary unit distinct from the bacterial chromosome (*5*). Possible factors associated with production of or sensitivity to pesticin II have not yet been investigated. The antibacterial activity of pesticin I, but not pesticin II, is strongly inhibited by ferric ions (*4*); and the virulence of wild-type *P. pseudotuberculosis*, like that of P^- strains of *P. pestis*, is strongly enhanced in mice treated with ferrous ion (*1*).

The role of pesticin I, coagulase, and the fibrinolytic factor in the pathogenic process has not yet been determined owing to difficulties of obtaining PI-deficient mutants that remain Pu^+, VW^+, and P^+. However, Burrows (*1*) found that a PI-negative strain of genotype Pu^+, VW^+, $F1^+$, and P^- was virulent in mice receiving ferrous ion. Thus, he suggested that pesticin I was either not essential for virulence or that ferrous ion fulfilled a dual requirement by replacing the products of both P^+ and PI^+.

Against this background, a report by Eisler (*6*) describing certain partially virulent strains of *P. pestis* as lacking coagulase was of interest, and Eisler kindly made these strains available. As expected, all strains lacked detectable pesticin I and the fibrinolytic factor; however, only strain G-32 proved to be both VW^+ and P^+. The genotype of strain G-32 is compared in Table 2 with those of wild-type *P. pestis* (strain Alexander) and *P. pseudotuberculosis* (strain PB1/+) with respect to established virulence determinants and other distinguishing properties. Table 3 shows the median lethal doses for these strains by three routes of injection in normal Detrick mice and for those receiving individual intraperitoneal injections of 40μg of ferrous ion.

The median lethal dose for strain Alexander was less than 10 cells in both treated and normal mice by all methods of infection. For intravenous injection, the dose for strains G-32 and PB1/+ was only slightly higher. These two strains showed reduced virulence after intraperitoneal infection in normal mice; high virulence could be restored by treatment with ferrous ion. Strain G-32 was avirulent when injected subcutaneously into both treated and control animals. Strain PB1/+ responded differently by this route, being partially and almost fully virulent in normal and treated mice, respectively.

These results demonstrate that pesticin I is associated with high virulence in normal mice infected by the intraperitoneal or subcutaneous route, but not by the intravenous route, and that ferrous ion does indeed fulfill a dual role in restoring virulence to mutants that are either P^- or fail to produce pesticin I. The PI-deficient strains possessed the potential for high virulence provided that physical barriers of the host were by-passed by intravenous injection. Evidently, production of pesticin I is associated with the normal ability to overcome these barriers, that is, the property of invasiveness. Further speculation concerning which gene products are critical and concerning the role of ferrous ion must be made with caution.

In general, bacteriocins are products of nonchromosomal genes that exist on cytoplasmic replicating units termed plasmids (*7*). Episomes are a related class of determinants, governing the expression of temperate bacteriophages, that are capable of integration on the chromosome or of autonomous existence in the cytoplasm (*8*). While extrachromosomal genes are seldom essential for bacterial growth, they may facilitate survival in special environments such as those encountered by pathogens. For example, certain bacteriophage genomes confer on their bacterial hosts the ability to produce toxins (*9*). Lysogeny can also result in antigenic changes that may facilitate survival of bacteria in otherwise immune mammalian hosts (*10*). In these cases, enhanced virulence is physiologically associated with toxin molecules or altered antigenic sites rather than with vegetative bacteriophages. By analogy, the products of *C* and *F* rather than *PI* would be associated with invasiveness in *P. pestis*. Nevertheless, it is conceivable (*5*, *11*) that pesticin I may possess siderophilin activity for *P. pestis* while

Table 1. Increase in median lethal dose (LD_{50}) for intraperitoneal injection, after loss of the established virulence determinants of *Pasteurella pestis* (*1*).

Virulence determinant				LD_{50}	
Pu	*VW*	*F1*	*P*	Mouse	Guinea pig
+	+	+	+	<10	<10
0	+	+	+	$>10^8$	$>10^9$
+	0	+	+	$>10^8$	$>10^9$
+	+	0	+	<10	$\sim10^4$
+	+	+	0	$>10^8$	$>10^9$

Table 2. Some properties (presence + and absence −) of *Pasteurella pestis* strain Alexander (wild type), *pestis* strain G-32, and *pseudotuberculosis* strain PB1/+ (wild type).

Pu	*VW*	*F1*	*P*	*PI*	*C*	*F*	*T**	*4**	*U**
Alexander									
+	+	+	+	+	+	+	+	+	0
G-32									
+	+	+	+	0	0	0	+	+	0
PB1/+									
+	+	0	+	0	0	0	0	+	+

* *T* = murine toxin; *4* = antigen 4; and *U* = urease. The significance of murine toxin and antigen 4 are discussed by Burrows (*1*).

Table 3. Results of determinations of the median lethal dose (LD_{50}) for *P. pestis* strain Alexander (Alex), *P. pestis* strain G-32, and *P. pseudotuberculosis* strain PB1/+ in normal and treated mice. The LD_{50} is expressed as number of cells; CL is 95-percent confidence limit as calculated by the method of Goldberg *et al.* (*15*). Immediately before challenge the $FeCl_2$ was injected intraperitoneally in suspension in 0.1 ml of peanut oil.

Strain	Results			
	No added Fe^{++}		Plus 40 μg Fe^{++}	
	LD_{50}	CL	LD_{50}	CL
		Intravenous injection		
Alex	8.1×10^0	4.9×10^0 to 1.5×10^1	9.8×10^0	5.6×10^0 to 1.5×10^1
G-32	7.1×10^1	4.0×10^1 to 1.4×10^2	2.3×10^1	1.3×10^1 to 4.2×10^1
PB1/+	3.9×10^1	2.2×10^1 to 6.6×10^1	3.1×10^1	1.6×10^1 to 5.7×10^1
		Intraperitoneal injection		
Alex	9.9×10^0	5.7×10^0 to 1.6×10^1	9.8×10^0	5.6×10^0 to 1.5×10^1
G-32	3.8×10^5	1.8×10^5 to 8.1×10^5	1.4×10^1	7.3×10^0 to 2.5×10^1
PB1/+	2.9×10^4	1.8×10^4 to 4.7×10^4	2.0×10^2	1.1×10^2 to 3.5×10^2
		Sub-cutaneous injection		
Alex	6.1×10^0	4.0×10^0 to 1.0×10^1	5.9×10^0	3.7×10^0 to 9.3×10^1
G-32	$>5.0 \times 10^8$		3.2×10^8	1.7×10^8 to 5.9×10^8
PB1/+	1.1×10^4	5.5×10^3 to 1.7×10^5	1.4×10^2	7.3×10^1 to 2.5×10^2

acting as a sideromycin for sensitive strains of *P. pseudotuberculosis*. This suggestion is attractive because *P. pestis* can grow slowly in nonhemolyzed serum whereas *P. pseudotuberculosis* does not. Both species grow rapidly after saturation of serum transferrin with ferrous ion (*12*). These results would be expected if *P. pestis* possesses a unique ability to obtain iron in vivo by chelation. However, the results shown in Table 3 indicate that loss or normal absence of this proposed activity is of little consequence to bacteria injected intravenously. An alternative suggestion is that strains lacking pesticin I are sensitive to a normal antibacterial component of serum that is inactivated by ferrous ion. An antirespiratory 7*S* globulin that fits this description has been described (*13*).

The major physiological determinant of virulence in *P. pestis* appears to be its ability to survive and multiply within phagocytes, especially within fixed macrophages of the reticuloendothelial system (*14*). Intracellular residence affords protection against normal antibacterial components of serum. The PI-deficient cell may fail to obtain a favored anatomical site in the mouse, suitable for intracellular growth, unless administered directly into the vascular system. The ability of wild-type *P. pestis* to obtain such sites after intraperitoneal or subcutaneous injection may be influenced by coagulase and fibrinolytic factor rather than pesticin I. To resolve the roles of these three factors completely, it may prove necessary to isolate PI^- strains that remain C^+ and F^+, or vice versa, by introduction of suitable point mutations on the determinant for pesticin I.

R. R. BRUBAKER
E. D. BEESLEY
M. J. SURGALLA

U.S. Army Biological Laboratories, Fort Detrick, Federick, Maryland

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Dynein: A Protein with Adenosine Triphosphatase Activity from Cilia

Abstract. *The adenosine triphosphatase protein from cilia of* Tetrahymena pyriformis *consists of 30S and 14S fractions. The 30S fraction consists of rod-like particles, 70 to 90 angstroms in diameter, which are linear polymers of globular 14S units. The 14S units have a molecular weight of approximately 600,000. The enzymatic properties of the two fractions are similar.*

Cilia isolated from *Tetrahymena pyriformis* contain a protein having adenosine triphosphatase activity (*1*). This protein forms the structures called "arms" which are located on the outer fibers of the cilium (*2*). Density-gradient fractionation (*2*) yields two protein fractions having adenosine triphosphatase activity. The fractions are characterized by their differing sedimentation rates (Fig. 1); extrapolated sedimentation constants ($s^0_{20,w}$) of 14*S* and 30*S*, respectively, have been determined in 0.1*M* KCl.

From our studies of this adenosine triphosphatase protein by physicochemical and enzymatic techniques, we conclude that the 14*S* and 30*S* fractions are related as monomer and polymer. We propose the term "dynein" (*dyne*, force; *-in*, protein) to describe this and other similar adenosine triphosphatase proteins associated with motile structures.

Electron micrographs of 30*S* dynein, shadow-cast with platinum by the method of Hall (*3*), show that it consists of rodlike particles of variable length (Fig. 2*A*). The heights of the particles (determined from shadow length) are 70 to 90 Å; the lengths, in a typical preparation, range between 400 and 5000 Å, 1700 Å for a particle of average weight. When the particles lie obliquely with respect to the direction of shadowing, a repeating globular structure (period approximately 140 Å) can often be seen along their lengths (Fig. 2*B*). Composite pictures, obtained by linear translation and superimposition (*4*) reveal this periodicity even more clearly (Fig. 2*C*).

Electron micrographs obtained by shadow-casting 14*S* dynein show globular particles (Fig. 2*D*). The heights are in the range 70 to 100 Å. Apparent widths are in the range 130 to 180 Å (Fig. 3); correction of the widths for lateral growth of shadowing metal (*3*) indicates the true width to be in the range 90 to 140 Å. Micrographs obtained by the negative-contrast method (*5*) show globular particles of essentially the same size (Fig. 2*E* and Fig. 3). Approximating the shape to an ellipsoid of axes 85, 90, and 140 Å, and assuming an anhydrous protein density of 1.35 g/cm^3, we calculate a molecular weight for 14*S* dynein of 540,000. The possible error in this value is estimated to be ±20 percent.

Molecular weights of several preparations of dynein have been determined by centrifugation. The "Archibald" method (*6*) yielded a value of 5,400,000 ± 1,000,000 for 30*S* dynein. The short-column equilibrium method (*6*) gave a value of 600,000 ± 100,000 for 14*S* dynein. Accuracy was limited by heterogeneity in each case: the 30*S* dynein is intrinsically polydisperse, whereas the preparations of 14*S* dynein contained

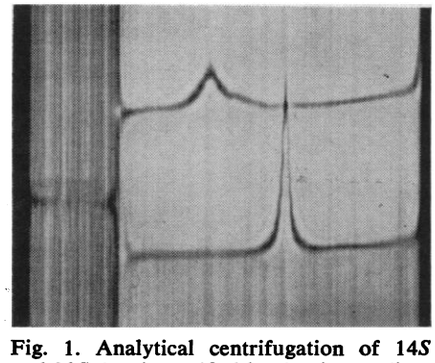

Fig. 1. Analytical centrifugation of 14*S* and 30*S* dynein purified by density-gradient centrifugation. Two cells were run, one with a 1° positive wedge-window to displace its trace upward. The lower trace shows 30*S* dynein (1.8 mg/ml); the upper trace shows 14*S* dynein (1.2 mg/ml). Solvent: 5 m*M* KCl, 0.1 m*M* ethylene diamine tetraacetate, 1 m*M* tris buffer, *p*H 8.2; speed, 59,780 rev/min in the Spinco model E analytical ultracentrifuge; direction of sedimentation is from left to right.