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Perspectives

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Toothpicks, Serendipity and the Emergence of the *Escherichia coli* DnaK (Hsp70) and GroEL (Hsp60) Chaperone Machines

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THE purpose of this essay is to retrace some of the early steps that I and a few (then) young geneticists took in the late 1960s and early 1970s to define the *Escherichia coli* functions used by phage to properly execute their developmental cycle. Eventually, this led to the discovery and functional understanding of the so-called DnaK (Hsp70) and GroEL (Hsp60) molecular chaperone machines, universally conserved among the biological kingdoms (Lindquist and Craig 1988; Georgopoulos and Welch 1993). Now we know that these and other molecular chaperone machines are involved in a multitude of biological processes, including protection of nascent polypeptide chains from premature aggregation, disaggregation of protein aggregates, polypeptide transport across biological membranes, and proteolysis (Bukau and Horwich 1998; Hartl and Hayer-Hartl 2002; Craig *et al.* 2006). Because protein denaturation and aggregation are enhanced by various environmental stresses, *e.g.*, an increase in temperature, it is not surprising that the DnaK (Hsp70) and GroEL (Hsp60) molecular chaperone machines were also discovered independently as "heat-shock" or "stress" proteins. Because of their very short growth cycles, phage have evolved a variety of strategies to subvert and customize host functions for their own use. Phage likely have a greater need than their hosts for quick and abundant chaperone power to carry out their developmental cycle in a timely fashion. If they fail to complete their cycle before the host lyses, infectious phage progeny will not be released into the medium, risking their extinction. This differential need for chaperone power likely explains why many of the bacterial mutations found to block phage development were eventually shown to be in genes encoding the GroEL and DnaK chaperone machines.

I dedicate this article to the memory of my friend Ira Herskowitz. Without his seminal contributions and insights, I never would have embarked on this type of research.

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THE (THEN) MYSTICAL WORLD OF PHAGE λ

Although my major at Amherst College was physics, I became interested in microbial genetics as a profession in 1961. As an undergraduate, I had taken a part-time job as a laboratory assistant working on phage P22 transduction in the laboratory of Harold H. Plough, a former student of T. H. Morgan. This experience persuaded me to pursue a Ph.D. with Salva Luria at the Massachusetts Institute of Technology (MIT). Under the steadying influence and guidance of Helen Revel (then a senior research associate in Luria's laboratory and a mother hen to me), I finished my Ph.D. thesis on the "sweet and sour" restriction of phage T4 (Revel and Luria 1970). From T4, I moved on to phage λ in Dale Kaiser's laboratory at Stanford. The reason for this leap from the T4 world to that of λ was largely due to the presence of Ethan Signer's laboratory down the hall at MIT. It seemed to me that at that time the smartest workers in biology were those working on phage λ. The developmental cycle and exquisite regulation of this organism are very complicated, and in those early days were way over my head. Thus, I decided to become part of the almost mystical and magical world of phage λ. I simply wanted to be as smart as those guys. It was going to be a great challenge.

Because of a temporary lack of bench space at Stanford, I decided to get a head start on my postdoctoral studies by finding a phage λ genetics project during my waiting

period at MIT. Luria supported my decision and generously arranged for me to remain as an Instructor in Microbiology for a few extra months. I have always loved genetics because of its strong reliance on logic. The isolation of mutants and the deciphering of their phenotypes allow the elucidation of concrete biological pathways and, in many instances, the identification of specific interactions among the various participants. Although genetic systems are elegant and powerful, they are also very exacting, occasionally yielding "unexpected" results that can be explained only when one fully understands the applied selection/screen.

IRA HERSKOWITZ AND THE DISCOVERY OF THE FIRST *E. coli groP* MUTANT

A key person during my initial ventures into lambdology was Ira Herskowitz, at that time a second-year graduate student in Signer's laboratory. Although he was a few years my junior, he was one of my phage λ intellectual fathers simply because he knew so much about it and was always friendly and available. In the spring of 1969, most λ workers were isolating and characterizing phage mutants (mostly of the nonsense/amber, or temperature-sensitive varieties) that were unable to propagate lytically, lysogenize, or recombine (Campbell 1993; Edgar 2004). During strategy-plotting sessions, Ira and I decided to take a completely backward approach, isolating and studying *E. coli* mutants that block the propagation of phage λ at a step subsequent to injection of its DNA. We reasoned that since the coding capacity of phage λ is rather limited, there must be many interesting host functions that directly or indirectly participate in its developmental cycle. Our hope was to uncover such protein–protein interactions between these two well-studied organisms, shedding additional light on the physiology of important functions in *E. coli*. Signifying our youthful independence was the fact that Ira and I never discussed our plans or specific experiments with our respective superiors. We simply forged ahead with our collaboration.

By serendipity, we chose to carry out our selection with the *E. coli* strain C600, carrying the *supE* amber (*am*) nonsense suppressor gene, an important detail whose significance will become obvious shortly. C600 bacteria were mutagenized and spread on LB agar plates along with an appropriate amount of phage λ*cI*$^-$ and phage 434*cI*$^-$ (Figure 1). These lambdoid phage variants were chosen because, although they grow well, neither of them can lysogenize, resulting in certain host death following infection. In addition, the fact that each attaches to a different host–surface receptor reduces the frequency of "trivial" bacterial mutants unable to adsorb phage, since both phage receptor-encoding genes would have to mutate to acquire resistance.

Normally, when a wild-type bacterium is infected, the released progeny phage will then infect neighboring

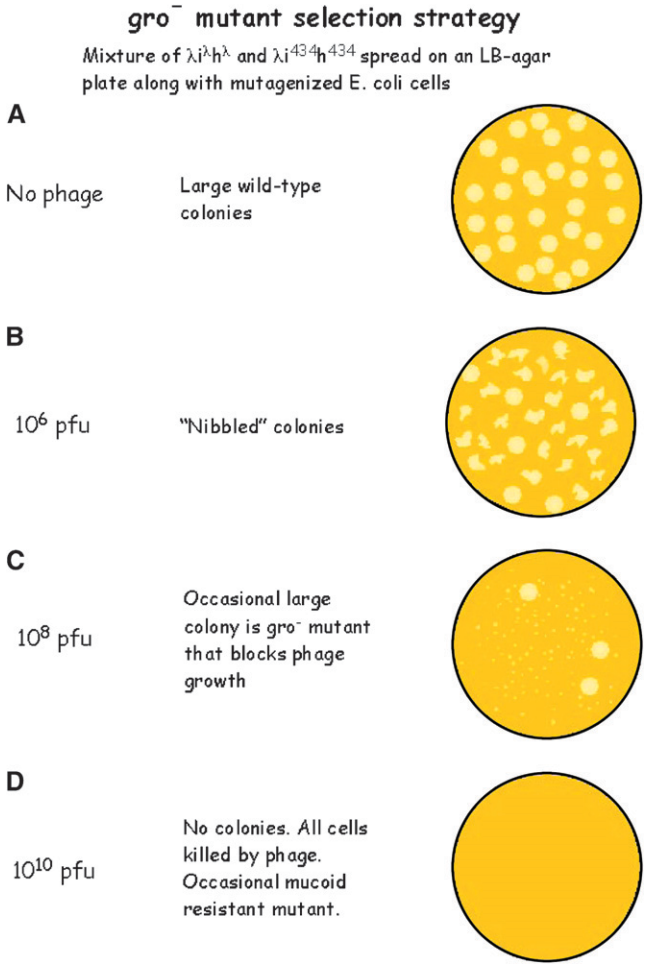

Figure 1.—The genetic strategy used to isolate the *E. coli* Gro mutants. The diagram shows the effect of increasing phage concentrations on bacterial growth and colony morphology. See text and Georgopoulos (1971) for details.

bacteria in the growing colony, thus limiting the size of the colony. For our experiment, the amount of phage mixed with the bacteria on the plate was critical, since too much phage kills all members of a growing colony (Figure 1D), whereas too little phage results in both "nibbled" colonies and many false positives (Figure 1B). If the infected bacteria are defective in a host function essential for phage development, no viable phage progeny will be released. Hence, although an individual bacterium may be killed as a result of phage infection, the infecting phage are eliminated in the process, thus sparing the neighboring sibling bacteria from being killed, and allowing colony formation (Figure 1C). The procedure was first carried out at 30° in the hope that these mutations would also block bacterial growth at higher temperatures. Under the chosen conditions, wild-type *E. coli* bacteria formed tiny colonies (Figure 1C) due to killing of the outer cells in a colony by the phage on the plate. However, at a frequency of $\sim 10^{-4}$, large-colony formers appeared on such plates (Figure 1C). The functions defined by these *gro* bacterial mutations

(named for their inability to *gro*w phage; this nomenclature was suggested to me later by Doug Berg at Stanford) did indeed turn out to be important for host growth, as exemplified by the *E. coli* Gro15 and GroC3 mutants (see below), originally isolated and studied at MIT, which are unable to form colonies at 43°.

PHAGE λ MUTANTS THAT COMPENSATE FOR THE *gro*15 BLOCK

While at MIT, Ira and I concentrated mostly on the *E. coli* mutant Gro15. Although the vast majority of λ*d*$^-$ phage are unable to form plaques on this strain, I noted the appearance, at an approximate frequency of 10^{-7}, of phage mutants that were able to somehow compensate for the *gro*15 block. Interestingly, none of the phage mutants isolated on Gro15 formed plaques on our other mutant, GroC3. Searching for clues as to their nature, I purified many of these *gro*15 compensatory phage mutants and tested them for growth on standard *E. coli* laboratory strains, including *supD*, *supE*, *supF* (*am*-suppressing), and various *sup*^{0} (nonsuppressing) wild-type bacterial hosts. To my great surprise and delight, ~20% of these λ compensatory mutants did not form plaques on any of the wild-type *sup*^{0} *E. coli* tested, but did grow on all of the various *am*-suppressing wild-type strains. Thus, these phage compensatory mutants carried a suppressible *am* mutation in some essential, yet unknown, phage gene. This finding highlighted the importance of starting with a strain carrying an *am* nonsense suppressor allele. Had we started with an *E. coli sup*^{0} strain, we would not have isolated such λ*am* compensatory mutants, consequently making it much more tedious to identify the corresponding λ suppressor gene.

I vividly recall our great anticipation and excitement as Ira tested Signer's laboratory collection of essential amber mutants of λ for complementation with our λ*am* compensatory mutants. The very simple, yet elegant technique of "spot" complementation enabled us, in <6 hr, to establish that all of our λ*am* mutants isolated on *E. coli gro*15 carried mutations in gene *P*, whose product was already known to be essential for λDNA replication. Because of my Greek origin, I baptized the λ compensatory mutants as "π" to indicate that they map in the *P* gene. We quickly showed that the *bona fide* λ*Pam*3 or λ*Pam*80 mutants of Allan Campbell also grew on the C600 *supE gro*15 mutant bacteria. Furthermore, recombination experiments showed that most of the λ*am* compensatory mutations were distinct from one another, as well as from *Pam*3 and *Pam*80.

Ira and I were perplexed as to how at least nine different *am* mutations in the λ*P* gene allowed the phage to grow on our C600 *supE gro*15 mutant host. Since C600 *supE gro*15 mutants do not grow at high temperature (Table 1), we knew that the corresponding *gro*15 gene function is essential for bacterial growth, at least at 43°.

TABLE 1

Plating properties of *groP* mutant bacteria

Bacteria	Growth at 43°	Plaque formation by phage		
		λ	λπA	λπB
groP$^+$ wild type	+	+	+	+
*groP*A15 (*dnaB*15)	–	–	+	+
*groP*B558 (*dnaB*558)	–	–	–	+
*groP*AB756 (*groPC*756; *dnaK*756)	–	–	+ or –	+

The original classification scheme devised for the *groP* class of bacterial mutants is depicted and is taken essentially from Table 1 of Georgopoulos and Herskowitz (1971), except that the later designations of the alleles are indicated in parentheses. The λπA or λπB phage compensatory mutants were isolated as plaque formers on the indicated *groP* bacterial mutants at an approximate frequency of 10^{-7} at 37° (see text for details). The subsequent genetic designation(s) of the bacterial alleles is given in parentheses.

Our preliminary conclusion was that, for λDNA replication, the λP protein must interact with an essential *E. coli* protein. Furthermore, we reasoned that our mutant host protein must be at least partially functional, since it carries out its essential bacterial function at all temperatures <43°. Perhaps by simply lowering the λP protein level (because *supE* is known to suppress *am* mutations only to ~5–30% of the wild-type levels), the partially disabled *gro*15 gene product manages to carry out its function more effectively, thus resulting in some viable phage progeny and hence in plaque formation.

THE PHAGE λP PROTEIN INTERACTS WITH *E. coli*'s DnaB PROTEIN

The phage P1-mediated transduction experiments of Ira at MIT (with help from Urs Kühnlein and Madeleine Jolit) showed that *gro*15 and the vast majority of the *groP* mutations isolated by me at Stanford mapped in or very near the *dnaB* locus of *E. coli*. This finding was rewarding because Fangman and Feiss (1969) had previously shown that λDNA replication was completely blocked in certain *dnaB* mutants. Furthermore, we showed that previously known *bona fide E. coli dnaB* mutants behave like our *groP* mutants, inasmuch as they preferentially support the growth of λπ mutants compared to wild-type λ. Thus, Ira and I specifically proposed at the 1970 Cold Spring Harbor phage λ meeting that, for successful λDNA replication, the host DnaB and phage λP proteins must interact (Figure 2). Later, Wickner (1979) proved and extended this interpretation by showing that the purified *E. coli* DnaB and phage λP proteins indeed form a complex and that all of DnaB's known biological activities are inhibited in this complex. While the preliminary results on the *groP* mutants that culminated from my collaboration with Ira were published only in the refereed Cold Spring Harbor

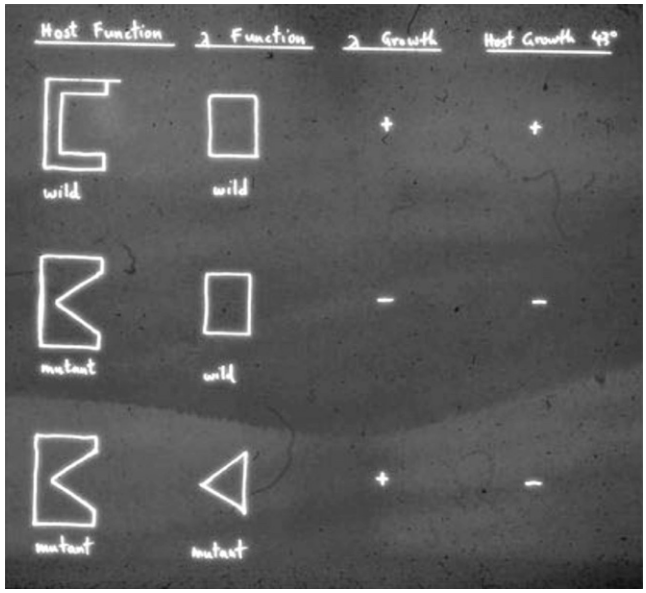

Figure 2.—The original diagram used by Ira and me during the 1970 Cold Spring Harbor meeting on phage λ to rationalize the apparent allele specificity observed between some of the *E. coli groP* mutants and the corresponding phage λP compensatory mutations.

Lambda I book edited by Al Hershey (Stahl 1998), I nevertheless consider this publication to be one of my very best (Georgopoulos and Herskowitz 1971).

THE DISCOVERY OF THE DnaK/DnaJ/GrpE CHAPERONE MACHINE

It turned out that the only mutation of my Stanford *groP* collection that did not map at or near the *dnaB* locus was *groP*AB756. Interestingly, *E. coli groP*AB756 exhibited the unique property of plating members of both the λπA and λπB compensatory mutant groups (Table 1). The *groP*AB756 mutation was eventually mapped during my subsequent stay in Harvey Eisen's laboratory in Geneva, Switzerland, at ~0.3 min of the *E. coli* chromosome, near the *thr* locus, and renamed *groPC*756 (Georgopoulos 1977). By using an *E. coli* DNA library cloned into a phage λ vector supplied by Barbara Hohn (Murray and Murray 1975), I was able to easily select for the λ*groPC*$^+$ recombinant through its ability to form normal-size plaques on the mutant *groPC*756 host (Georgopoulos 1977). The fact that the *groPC*756 (λ*groPC*$^+$) lysogen supports hetero-immune lambdoid phage growth and forms colonies at high temperature proved that the *groPC*756 mutation is recessive to the wild-type allele and solely responsible for both bacterial temperature sensitivity and the block to lambdoid phage growth. I also concluded that most likely the GroPC protein forms a complex with the λP protein and somehow this interaction is vastly weakened by the *groPC*756 mutation.

At about this time, Mike Feiss's group had independently isolated an *E. coli* mutant, GroPC259, on the basis of its inability to propagate both phage λ and phage P2. In their study, published in the same issue as Georgopoulos (1977), Sunshine *et al.* (1977) showed that their *groPC*259 mutation is indeed very closely linked to *groPC*756 and, like *groPC*756, also affects bacterial growth at high temperature.

Saito and Uchida (1977), selecting for growth of an appropriate λ-defective lysogen at 42°, also isolated bacterial mutations that interfere with λDNA replication and named these mutations *grp* (*gro*P-like). One of their classes, *grpA*, was located in the *dnaB* gene, while another, *grpC*, mapped near the *groPC756* and *groPC*259 alleles. Ira played a catalytic role in the ensuing collaboration with the Feiss and Uchida laboratories, which quickly established that all of the *groPC* or *grpC* alleles indeed fell into two adjacent but distinct complementation groups (Yochem *et al.* 1978) defined by *groPC*756 and *groPC*259. Saito and Uchida (1977, 1978) renamed the genes *dnaK* and *dnaJ*, respectively, because they affect host DNA synthesis at high temperatures. Two members of one chaperone machine had been identified.

The third member of the DnaK/DnaJ/GrpE chaperone machine was identified on the basis of genetic properties of the *grpE*280 mutation of Saito and Uchida (1977), which maps at 56 min (Saito *et al.* 1978). The GrpE280 mutant was isolated as a colony former at 42°. However, subsequent transductional analysis showed that *E. coli* GrpE280 mutant cells do not grow at >43° and that the *grpE* gene is essential even for bacterial growth at all temperatures (Ang *et al.* 1986; Ang and Georgopoulos 1989). Although the *grpE*280 mutation also blocks host DNA synthesis at 43°, its gene designation was not changed (Ang *et al.* 1986).

THE ROLE OF THE DnaK/DnaJ/GrpE CHAPERONE MACHINE IN λDNA REPLICATION AND PROTEIN DISAGGREGATION

The exact role of the DnaK/DnaJ/GrpE chaperone machine in λDNA replication was deciphered over the next several years in various laboratories, including that of Maciej Zylicz (in collaboration with our laboratory), Roger McMacken, the late Hatch Echols, and Sue Wickner (reviewed in Ang *et al.* 1991). The key step in both *E. coli* and λDNA replication is the correct positioning of the essential *E. coli* DNA helicase DnaB at a unique chromosomal site, called *oriC* in *E. coli* and *ori*λ in phage λ. To assist DnaB positioning on λDNA, the λO replication protein binds specifically to *ori*λ, located within the *O* structural gene itself (Furth *et al.* 1978). The phage λP protein, behaving as Dr. Jekyll, then binds DnaB helicase and specifically delivers it to the *ori*λ site by simultaneously interacting with the λO protein (Furth *et al.* 1978; Zylicz *et al.* 1984). However, the Mr. Hyde side of λP's character manifests itself by suppressing

all known activities of DnaB in this complex (Wickner 1979). Thus, although the DnaB helicase has been correctly positioned at *ori*λ, it cannot unwind the DNA until λP is removed from the complex. This is accomplished through disaggregation of the λO–λP–DnaB complex at *ori*λ by the DnaK/DnaJ/GrpE chaperone machine, resulting in the release of λP and the initiation of DNA replication (reviewed in Ang *et al.* 1991).

Shortly after, it was shown that the DnaK chaperone machine also disaggregates heat-inactivated *E. coli* RNA polymerase (Skowyra *et al.* 1990), as well as aggregated DnaA protein, whose monomerization is essential for bacterial DNA replication (Hwang *et al.* 1990). This finding provides the rationale for why *dnaK*, *dnaJ*, or *grpE* mutations interfere with bacterial DNA replication at high temperature.

One of the ways in which the DnaJ and GrpE proteins assist the DnaK chaperone to carry out its biological function is by accelerating its very slow ATPase activity. DnaJ specifically accelerates the hydrolysis of DnaK-bound ATP, while GrpE accelerates DnaK's ATPase cycle by acting at the level of nucleotide release (Liberek *et al.* 1991; Bukau and Horwich 1998; Hartl and Hayer-Hartl 2002). DnaJ also facilitates DnaK's chaperone chores by binding and "presenting" specific substrates to DnaK (reviewed in Craig *et al.* 2006). In this respect, it is interesting to note that the original *dnaJ*259 and *grpE*280 mutations specifically interfere with the interaction of their corresponding gene products with DnaK (Johnson *et al.* 1989; Liberek *et al.* 1991; Keppel *et al.* 2002; Kerner *et al.* 2005), whereas the original *dnaK*756 mutation interferes with the interaction of the DnaK756 protein and GrpE (Georgopoulos *et al.* 1972, 1973; Johnson *et al.* 1989; Buchberger *et al.* 1996).

THE DISCOVERY OF THE GroEL/GroES CHAPERONE MACHINE

The discovery of the *E. coli* genes encoding the GroEL chaperone machine was made in the early 1970s, again through the efforts of bacteriophage geneticists isolating *gro*-type bacterial mutants that blocked either phage λ (Georgopoulos *et al.* 1972, 1973; Sternberg 1973) or phage T4 (Takano and Kakefuda 1972; Coppo *et al.* 1973; Revel *et al.* 1980). My studies on *groE* at Stanford were carried out in collaboration with a fellow postdoc, Roger Hendrix. Roger played an important role in both the early stages and the subsequent development of the GroEL chaperone machine story (see below). Again, serendipity played key roles in the GroE story. For example, 30% of the λ compensatory mutants isolated at Stanford as plaque formers on GroC3, isolated at the same time as Gro15, had either *am* or temperature-sensitive mutations in the capsid-encoding gene *E* of λ (originally referred to as λε). Thus, the GroC3 mutant was renamed GroEAC3 to designate this fact.

TABLE 2

Plating properties of *groE* mutant bacteria

Bacteria	Growth at 43°	Plaque formation by phage				
		λ	λεA	λεB	T4	T4ε1
groE^{+} wild type	+	+	+	+	+	+
*groEA*SC3 (*groES*C3)	−	−	+	−	+	+
*groEA*44 (*groEL*44)	−	−	−	−	−	+
*groEB*515 (*groEL*515)	+	−	−	+	+	−

The original *E. coli groE* mutant isolates were arbitrarily divided into classes A or B on the basis of the ability of the various λε compensatory mutations to form plaques on them. It turned out later that all *groES* mutations belonged to the *groEA* group, while *groEL* mutations fell into both the *groEA* and the *groEB* groups (new designation given in parentheses).

The various *groE*-type mutants in my Stanford collection were arbitrarily classified as GroEA or GroEB on the basis of their ability to propagate various λε compensatory mutants (Table 2). I observed that members of both the *groEA* and the *groEB* classes did not propagate other lambdoid phage (nor did the virulent phage T5) and interfered with bacterial growth at high temperature. A subsequent detailed genetic analysis of λε missense mutants revealed that whereas the λεA mutations always mapped in the *E* gene, the λεB mutations mapped in either the *E* or the *B* gene, required for correct morphogenesis of the phage capsid. This result was gratifying because Kaiser's electron micrographs of *groE* bacteria infected by wild-type λ clearly showed that the λE capsid protein was assembled, but in an aberrant manner (Georgopoulos *et al.* 1973). In this respect, infection of *groE* bacteria by wild-type λ resembled infection of wild-type *E. coli sup*^{0} bacteria by either λ*Bam* or λ*Cam* phage. Thus, it appeared from those early studies that the block exerted by our *groE* mutations on phage λ assembly was very likely at the level of λB action. In this respect, the *groE* designation is a misnomer, and the name *groB* would have been more appropriate! Kochan and Murialdo (1983) later showed that the GroEL machine proteins indeed play a primary role in the proper assembly of the λB dodecameric structure, an early step in λ prohead assembly. All of the above observations left the long-standing impression that the GroEL machine's primary role is the correct assembly of macromolecular structures. It would have been almost impossible to realize in the early 1970s that the GroE proteins act uniquely at the level of folding single nascent polypeptide chains, and that their subsequent assembly into macromolecular structures follows spontaneously (see Figure 3 and below).

The second lucky finding was the observation that one of the *E. coli* mutant hosts, GroEA44, in addition to not propagating λ, was unique among my isolates in not propagating my old friend phage T4 as well, although I had exerted no selection for such a phenotype in the

original isolation procedure (Table 2). However, again, at a frequency of $\sim 10^{-7}$, T4 compensatory plaque-forming mutants (originally referred to as T4ε) could be isolated. The third stroke of luck was that one of these phage T4 mutants, ε1, simultaneously lost its ability to propagate on some of the other *groE* mutant hosts (*e.g.*, *groE*B515), which otherwise allow normal growth of wild-type T4 phage (Table 2). The inability of T4ε1 to grow on the GroEB515 mutant host led to the quick assignment of its mutation to the morphogenetic gene *31*, again by spot complementation tests using a battery of known T4 *am* mutants (in collaboration with William Wood, then at Caltech) (Georgopoulos *et al.* 1972). In this respect, T4-infected GroEL mutants exhibit a phenotype identical to wild-type *sup*^{0} bacteria infected by T4 *31 am* mutants (Laemmli *et al.* 1970). The effect of a single mutation on both λ and T4 phage head assembly pointed to a possible direct interaction between the *groE*A44 gene product and the T4-encoded *31* gene product, Gp31, in proper protein assembly. Essentially similar conclusions about Gp31/GroEL interactions were reached by Takano and Kakefuda (1972), Coppo *et al.* (1973), and Revel *et al.* (1980), who named their corresponding *groE*-like mutations *mop*, *tabB*, and *hdh*, respectively.

HOST- AND PHAGE-ENCODED GroEL CO-CHAPERONES

By using the first phage λ recombinant libraries of *E. coli* DNA, λ*groE*$^+$-transducing phage were easily selected as plaque formers on various *groE* mutant hosts (Georgopoulos and Hohn 1978; Hendrix and Tsui 1978). Barbara Hohn played a catalytic role in the isolation of a λ*groE*$^+$-transducing phage by obtaining the λ library from Ken Murray, whereas Hendrix independently obtained his library from Ron Davis's laboratory. Both groups easily identified the "GroE" product as an ~60,000-Da protein following infection of UV-irradiated bacteria. The easy overproduction of "GroE" from λ*groE*$^+$-infected cells led to its purification by Hendrix (1979) and Hohn *et al.* (1979), followed by determination of its large, tetradecameric structure and ATPase activity.

Subsequently, a more careful genetic analysis of various λ*groE*$^+$-deleted derivatives led us to the realization that there are two *groE* cistrons, named *groEL* (to signify the *l*arge 60,000-Da GroEL polypeptide) and *groES* (to indicate the *s*mall ~15,000-Da polypeptide) (Tilly *et al.* 1981). Approximately half of my *groE* mutant alleles mapped in the *groEL* gene and the rest mapped in *groES*. Purification of GroES demonstrated its ATP-dependent interaction with GroEL (Chandrasekhar *et al.* 1986), and its negative modulation of GroEL's ATPase activity. These biochemical results were anticipated by earlier genetic suppressor studies (Tilly and Georgopoulos 1982), and they helped explain why mutations in either *groES* or *groEL* exert the same phenotypes on the growth of *E. coli* or phage λ (Georgopoulos *et al.* 1973). The genetic experiments of Olivier Fayet clearly demonstrated that the *groES* and *groEL* genes are the only major chaperone-encoding genes absolutely essential for *E. coli* viability under all conditions tested (Fayet *et al.* 1989). This essentiality has been traced to its unique ability to fold a small number of essential *E. coli* proteins (Kerner *et al.* 2005).

It took a few more years to demonstrate directly that Gp31 is a *bona fide* GroEL co-chaperone (van der Vies *et al.* 1994; Richardson *et al.* 1999). Further studies showed that many large bacteriophage encode Gp31-like proteins capable of substituting for GroES in *E. coli* growth (reviewed in Ang *et al.* 2000, 2001; Keppel *et al.* 2002). However, it is still not clear why Gp31, but not GroES, is uniquely required for correct folding of the Gp23 capsid protein.

It is interesting to point out again that the use of phage λ in our original selection was serendipitous because it led to the isolation of mutations in both *groES* and *groEL*, thus revealing the existence of the two genes. If we had used only phage T4, which does not need GroES, only *groEL* would have been identified under these circumstances.

Because of the GroE chaperone machine's effect on phage morphogenesis and the fact that Hsp60, the eukaryotic GroEL homolog, is also involved in the correct assembly of the large, oligomeric Rubisco protein in plants, the notion that GroE is uniquely involved in macromolecular protein assembly persisted until 1988 (Hemmingsen *et al.* 1988). This concept began to unravel when Bochkareva *et al.* (1988) clearly demonstrated that GroEL crosslinks in crude extracts to the nascent chain of β-lactamase, a monomeric enzyme destined for the *E. coli* periplasm. Shortly after, George Lorimer's group demonstrated that the GroES/GroEL chaperone machine assists the correct folding of prokaryotic Rubisco in a purified system (Goloubinoff *et al.* 1989). The ensuing years resulted in an explosion in our knowledge of the mechanistic details of the GroES/GroEL machinery (Bukau and Horwich 1998; Hartl and Hayer-Hartl 2002).

THE DnaK AND GroEL CHAPERONE MACHINES AND INTRACELLULAR PROTEIN FOLDING

Protein folding, protein aggregation, and protein–chaperone interactions are highly dynamic processes (Bukau and Horwich 1998; Hartl and Hayer-Hartl 2002; Bukau *et al.* 2006; Chiti and Dobson 2006; Craig *et al.* 2006). Figure 3 outlines the role of the DnaK and GroEL chaperone machines in *E. coli* to prevent premature nascent polypeptide aggregation, thus promoting proper folding. Trigger Factor (TF; the *tig* gene product) is a highly abundant chaperone that reversibly binds to the ribosome in the vicinity of the polypeptide

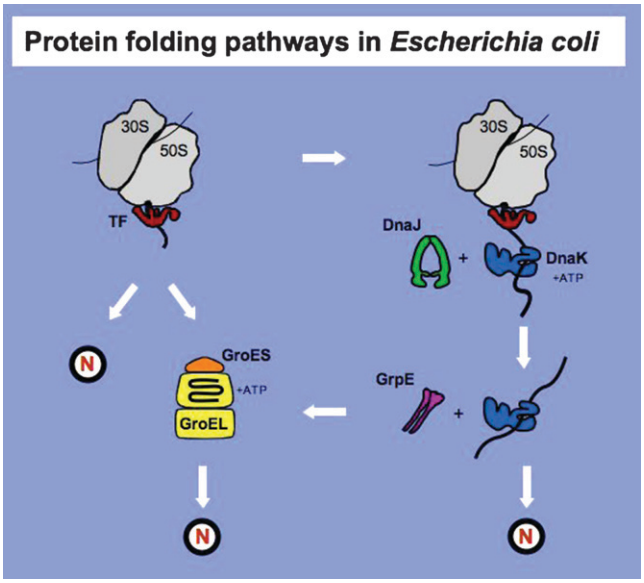

Figure 3.—Role of DnaK and GroE chaperone machines in nascent protein folding. The diagram depicts the ribosome, the ribosome-bound TF chaperone, and the DnaK/DnaJ/GrpE and GroES/GroEL chaperone machines. The diagram depicts the various pathways that nascent polypeptides may follow during the intracellular folding process to arrive at their native state (N). It is essentially adapted from Hartl and Hayer-Hartl (2002), except all structures are drawn to scale. See text for details.

channel exit and thus interacts first with the emerging nascent chains. Subsequently, the DnaK chaperone machine can step in and bind some of these nascent chains. However, because of their substrate-binding promiscuity, additional chaperones may also interact transiently, and with varying affinities, with a given protein substrate. The key role that the TF and DnaK chaperone machines play in protein folding is highlighted by the resulting synthetic lethality of the *tig dnaK* double mutant above 30° (Genevaux *et al.* 2004; Vorderwulbecke *et al.* 2004). The DnaK (Hsp70) machine, often in collaboration with the ClpB (Hsp104) chaperone, can also disaggregate certain protein aggregates once formed (Bukau *et al.* 2006). In contrast to TF and the DnaK machine, the GroEL machine is generally thought to act mostly at a post-translational level to help some polypeptides fold properly. The potential *in vivo* interchangeability and plasticity of the various chaperone machines is exemplified by several recent findings. First, Ying *et al.* (2006) have shown that GroEL can unexpectedly associate cotranslationally with nascent chains in a well-defined *in vitro* system. Second, the overproduction of either the GroEL machine or the SecB chaperone enables both growth of the *tig dnaK* double mutant and a reduction of protein aggregates at otherwise lethal temperatures (Genevaux *et al.* 2004; Ullers *et al.* 2004; Vorderwulbecke *et al.* 2004). Third, both the *E. coli* DnaK chaperone machine and its various eukaryotic Hsp70 homologs can function interchangeably both *in vivo* and *in vitro* with various DnaJ-like and GrpE-like factors (Brodsky and Chiosis 2006; Craig *et al.* 2006). Thus, it is likely that *in vivo* some nascent polypeptide chains can be "cradled" by more than one chaperone machine "midwife."

Recent studies have also highlighted the potential role of the Hsp70 chaperone machine in modulating a variety of important cellular functions, such as apoptosis, tumor growth, and certain human diseases caused by protein aggregation, *e.g.*, Parkinson's and Huntington's diseases. In turn, this has spurred the development of small molecule modulators of Hsp70 activity as therapeutic agents (Brodsky and Chiosis 2006). The Hsp60 human chaperone machine has also been shown to play an important role in the development of a particular form of human spastic paraplegia (Hansen *et al.* 2002).

EPILOGUE

Ira visited Geneva a few months before his untimely death in the spring of 2003 (Botstein 2004). One evening we took a long walk along the beautiful lake shore and reminisced about the "good old days." We both felt lucky that we lived through the relatively early stages of the 1953 "big bang" era of molecular biology. In the spring of 1969, armed with sterile toothpicks, we embarked on a long journey of adventure and discovery but, unlike Odysseus, with no clear destination in mind. We were heartened that our genetic studies, with such primitive tools as toothpicks and phage spot tests, had contributed to a deeper understanding of the intracellular protein-folding process. We were certain that plenty of important discoveries were still to be made, hiding at the tips of toothpicks wielded by future generations of microbial geneticists. Finally, we agreed that genetic analyses of simple organisms, such as phage, bacteria, and yeast, is the easiest and fastest way to arrive at "Hershey's Heaven" (Stahl 1998).

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