FEBS Letters 402 (1997) 4–8 FEBS 18085

Hypothesis

Does selective gene activation direct evolution?

Barbara E. Wright*

Division of Biological Sciences, The University of Montana, Missoula, MT 59812-1002, USA

Received 28 October 1996

Abstract Mechanisms may have evolved such that the unique metabolic reaction to a particular environmental stress results in higher mutation rates of those genes most likely to solve the problem. Evidence is presented indicating that the environment in effect directs the evolution of organisms by (1) presenting various kinds of stress resulting in metabolic activities that target particular genes for increased rates of transcription and mutation, and (2) selecting among this specifically enriched mutant population those variants that alleviate the imposed stress. This process should be ongoing and would be expected to accelerate the rate of microbial evolution.

Key words: Stringent response; Derepression; Guanosine tetraphosphate; Stress

1. Introduction

Over the years there have been many speculations as to whether evolution is somehow directed, or can be explained purely as a result of random mutations and natural selection. A number of investigators believe that evolution happens too fast: if every random mutation has to be tested against the environment for selection or rejection, there would not have been enough time to evolve the superbly ordered biochemistry we see in organisms today [1]. Therefore, some non-random mechanisms must guide and accelerate the process in selected directions. There is in fact evidence from marine fossil communities that environmental stress accelerates the rate of evolution [2]. The distribution of these communities across the continental shelf, from the most modern (360 million years old) to the oldest (500 million years old), are strongly time-progressive, indicating that major new faunal associations originate in the nearshore environments and then spread outwards across the shelf. Species diversity is higher in outer-shelf, offshore communities than in nearshore communities. Yet it is in the species-poor, stressed, nearshore communities that the most recently evolved forms of life exist. In cold, species-poor, stressed Arctic latitudes there is also a preferential origination of novel plant and animal species, which then spread south [3].

This review examines the relationships between nutritional stress, transcription, mutation and evolution. In the starvation systems to be described, a pattern of gene activation occurs in response to a particular kind of nutritional stress that is then relieved by the enzymes that are synthesized. For example, enzymes in an amino acid biosynthetic pathway become derepressed in the absence of their end product. The process of transcription increases the concentration of single-stranded DNA that is especially vulnerable to mutagenesis. It follows that specific starvation regimens resulting in particular patterns of transcription will also enhance mutation rates in those genes that become activated.

2. The link between starvation regimens and patterns of transcription

In all likelihood, evolving microorganisms in nature rarely, if ever, enjoy optimal nutritional conditions and maximal rates of cell division. As their metabolism is usually that of starvation, macromolecular synthesis is inhibited and potential sources of nutrition are activated, such as proteolysis, or the accumulation of catabolic, amino acid biosynthetic or other enzymes that could alleviate the particular state of partial starvation. The mechanisms that have evolved for activating the transcription of genes encoding enzymes essential to continued viability under different conditions of nutritional stress are most ingenious. Under laboratory conditions, such gene activation usually is initiated during the transition from logarithmic growth into the stationary phase. For example, such a transition occurs in enteric bacteria when the concentration of ammonia falls below 1 mM. Above this level, the cells assimilate nitrogen by aminating α-ketoglutarate to glutamate in a reaction catalyzed by glutamate dehydrogenase. As ammonia levels become limiting, there is an increase in the rate of transcription of glutamine synthetase which has a higher affinity for ammonia, thus alleviating the problem [4]. Phosphate deprivation is relieved in much the same manner. When inorganic phosphate levels fall below 1 mM, the constitutive low-affinity phosphate transport system with a K_m of 25 mM is supplemented with an induced system having a K_m of 0.16 mM [5]. By mechanisms such as these the nutritional status of the environment constantly fine-tunes the transcriptional machinery of the cell. Thus, when the cell is deprived of an essential amino acid, the pattern of activation of various genes for amino acid biosynthetic enzymes depends upon the severity of the need for, and upon the supply of, all the other amino acids [6–8]. In minimal medium, the relative expression of the *his* operon varies from a derepressed level of 12 to a repressed level of 1; in rich medium the operon can be further repressed to the level of 0.25 [7].

Two critical signal nucleotides that regulate the metabolism of starvation are cyclic AMP (cAMP) and guanosine tetraphosphate (ppGpp). Cyclic AMP complexed with the catabolite activator protein (CAP) binds to a particular consensus sequence, thereby activating a number of promoters involved in carbon metabolism. During carbon source starvation, cAMP as well as ppGpp may accumulate. While these super-control molecules primarily regulate different metabolic do-

*Corresponding author. Fax: (1) (406) 243-4184.
E-mail: bewright@selway.umt.edu

PII S0014-5793(96)01479-2

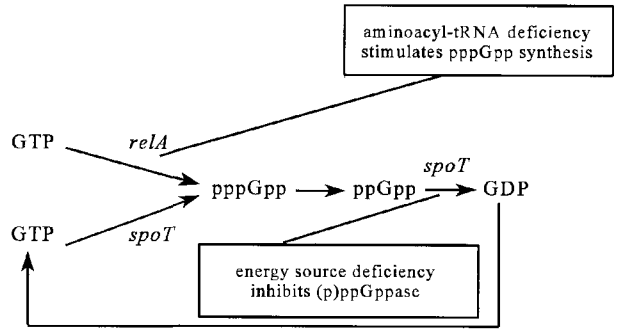

Fig. 1. The (p)ppGpp metabolic cycle. The synthesis of pppGpp from GTP is catalyzed by the *relA* gene product, ppGpp synthase I, which is a pyrophosphoryltransferase reaction using ATP as the donor and GTP as acceptor. The (p)ppGpp 3′-pyrophosphohydrolase activity is catalyzed by the *spoT* gene product.

mains, their areas of control also overlap in a complex hierarchy of regulation [7]. The synthesis of β-galactosidase in vitro is strongly dependent upon both cAMP and ppGpp [9].

The metabolic reaction of *Escherichia coli* to starvation for amino acids is called the stringent response, which inhibits rRNA, tRNA and cell wall synthesis while activating catabolic and amino acid biosynthetic operons [6]. If an uncharged tRNA correctly pairs with the mRNA codon exposed at the A site of the ribosome, an 'idling reaction' is established in which GTP and ATP form pppGpp which is then converted to ppGpp (Fig. 1). This reaction is catalyzed by ppGpp synthase I, the *relA* gene product. Guanosine tetraphosphate levels are also controlled by a degradative enzyme, (p)ppGpp 3′-pyrophosphohydrolase, the *spoT* gene product. Stringently controlled or inhibited promoters (e.g. those for rRNA and tRNA synthesis) contain a GC-rich discriminator sequence to which RNA polymerase binds; ppGpp interferes with this binding and prevents transcription [10]. In contrast, the discriminator regions of amino acid biosynthetic operons are AT-rich, with sequences that do not favor the binding of RNA polymerase and open complex formation. When ppGpp binds to RNA polymerase a conformational change occurs that allows binding to DNA followed by transcription. Among those operons activated by ppGpp, there is additional specificity with respect to the concentrations of ppGpp that are optimal for their activation [9,11,12]. For example, the *lac* operon requires 3–4 times more ppGpp than the *his* operon for either optimal or half-maximal expression [9]. Moreover, starvation for different amino acids results in the accumulation of different concentrations of ppGpp [13]. This intriguing observation may be related to the tRNA abundance for different amino acids [14,15].

In yeast the stringent response to amino acid starvation has been elucidated in elegant detail [8,16]. As in *E. coli*, the accumulation of uncharged tRNA triggers the response; however, ppGpp is apparently not involved in this system. Rather, the uncharged tRNA stimulates the activity of a protein kinase (GCNZ) that phosphorylates a translation initiation factor that, in turn, indirectly induces the expression of a transcription factor, GCN4, about 10-fold. Transcription is activated in at least 40 different genes encoding amino acid biosynthetic enzymes that are derepressed 2–10-fold. For each enzyme subject to this general control, derepression was demonstrated by starvation for an amino acid whose biosynthesis does not depend upon that enzyme. Superimposed upon this general control system are independently controlled operon-specific regulation mechanisms [17]. In both yeast and *E. coli* it is thus apparent that the pattern of increased transcriptional activity resulting from a given starvation regimen is highly evolved and finely tuned to alleviate the particular condition of stress imposed by the environment.

3. The link between transcription and mutation rates

The intrinsic chemical instability of nucleic acids can result in significant background ('spontaneous') mutation rates [18]. Hydrolytic reactions are observed under physiological conditions, and occur at a lower rate in double-stranded than in single-stranded DNA, e.g. deamination of C→U is increased more than two orders of magnitude in single-stranded DNA [19]. It is therefore not surprising that mutagens are most damaging during transcription, when single-stranded segments of DNA are exposed. Herman and Dworkin [20] observed that, during exponential growth of *E. coli*, *recA*-independent *lac*$^-$ reversions caused by a chemical mutagen were stimulated in the presence of the *lac* inducer. In contrast, the frequency of *lys*$^-$ reversions was not affected by IPTG. Similar investigations of the *lac* system by Brock [21] demonstrated that three alkylating agents enhanced reversion rates 10–30-fold in induced compared to non-induced cells. Reversion rates of mutants associated with ineffective regulatory systems (*i*$^-$*o*$^+$) were not differentially affected by IPTG induction. In other studies, Cordaro and Balbinder [22] found increased background reversion rates of tryptophan mutants in strains with a constitutively derepressed *trp* operon, and UV-induced reversions of *hisC*$^-$ and *hisF*$^-$ mutants were increased 5–8-fold under conditions producing a 15-fold derepression of the *his* operon [23]. Again, the latter effect was specific in that the mutability of an unlinked streptomycin locus was not affected. Also, although *his*$^-$ mutants deficient in excision repair showed increased reversion frequencies in both repressed and derepressed strains, their relative ratio remained constant. These early investigations have recently received strong support by a clear demonstration of the dependence of background mutation rates on transcription [24]. A yeast chromosomal *lys2*$^-$ frameshift allele was placed under transcriptional control of an inducible promoter, and a 35-fold increase in reversion rate occurred when the *lys2*$^-$ allele was transcribed at a high level. Reversion rates of an uninducible *lys2*$^-$ allele were not affected. A fascinating example of derepression in the evolution of a catabolic pathway has been described in *Aerobacter aerogenes* [25]: in the presence of xylitol, derepression of ribitol dehydrogenase led to mutations that produced an altered enzyme with higher catalytic activity as a xylitol dehydrogenase! It should be pointed out that the mutations

Table 1
A summary of *malA*$^-$ reversion rates in glycerol-starved isogenic strains CP78 (*relA*$^+$) and CP79 (*relA*$^-$)[a]

	CP78	CP79
Total cell number × 10^8	1.0 ± 0.14	0.91 ± 0.25
P_0 (no. negative plates/total)	0.67 ± 0.15	0.77 ± 0.06
Mutation rate × 10^{+9}	0.27 ± 0.14	0.21 ± 0.07

[a]Values given are averages of 5 mutation rate experiments performed as described previously [31,32]. Cultures were grown with limiting glycerol (4.0 mM) and plated to minimal medium containing maltose as the sole carbon source.

discussed in this review occur during or at the end of growth, in contrast to adaptive mutations that occur after prolonged starvation in non-growing, stationary-phase cells.

An effect of transcription on mutation rates is consistent with its effect on the mechanisms of DNA repair: single-stranded DNA is especially vulnerable to damage and, by the same token, more accessible to the mechanisms of repair. In *E. coli*, RNA polymerase stalled at a damaged site is a signal for intervention by the transcription repair coupling factor, which first binds to the area of the lesion and then recruits the nucleotide excision repair enzymes that preferentially act on the transcribed strand [26,27]. SOS mutagenesis also involves transcriptional derepression; single-stranded DNA is the primary signal for SOS induction in vivo [28,29]. The very process of repairing lesions resulting from the intrinsic instability of DNA and from naturally occurring mutagens in the environment may contribute to the rate of background mutations.

Finally, a variety of evidence implicates both transcriptional and repair machinery in the site-directed hypermutation process of immunoglobin genes in response to challenge with antigen [30].

4. The link between mutation rates and the stringent response

Given the relationships between nutritional stress, transcription and mutations, one might predict that any starvation regimen provoking a specific pattern of transcriptional activity would also show enhanced mutation rates in those genes that had become active. In the case of the stringent response in *E. coli* K12, it has been possible to substantiate this prediction using two isogenic multiple auxotrophs differing only in *relA* [31]. When starved for an amino acid, reversion rates of *leuB*$^-$ and *argH*$^-$ alleles were significantly higher in the *relA*$^+$ than the *relA*$^-$ strain. This effect of allelic differences in *relA* alone on reversion rates implicates transcription and the ppGpp system; fully derepressed transcription depends upon the *relA*$^+$ genotype [6,7]. The distribution of *leu*$^+$ revertants in mutation rate experiments indicated that they occurred predominantly after exponential growth had ceased, when the classical stringent response begins with a burst in the concentration of ppGpp. At the end of growth and during exponential growth, under a variety of conditions, ppGpp levels were correlated with reversion rates of the *relA*$^+$ and *relA*$^-$ strains [31,32]. The specificity of enhanced mutation rates resulting from the stringent response is illustrated by the observation that starvation for threonine as well as leucine increased reversion rates of the *leuB*$^-$ allele in the *relA*$^+$ compared to the *relA*$^-$ strain; starvation for these two amino acids results in the accumulation of comparable levels of ppGpp [13]. At present it is difficult to predict whether starvation for a particular amino acid will affect the rates of transcription and mutation of a particular operon. For example, the increase in ppGpp levels provoked by starvation for arginine may enhance mutation rates of the normal and abnormal genes of the *arg* operon, yet not affect the *his* operon that is already maximally activated by endogenous ppGpp levels.

Two important controls were carried out that substantiate the specificity of the stringent response in stimulating derepression and mutation rates in amino acid biosynthetic pathways. First, how does starvation affect mutations that do not depend on the *relA*$^+$ genotype? Second, how does amino acid starvation affect mutations of genes not in amino acid biosynthetic pathways? *RelA*$^+$-mediated ppGpp synthesis is known to respond to amino acid starvation but not to carbon source deprivation, whereas *spoT*-mediated ppGpp accumulation responds to carbon source deprivation but not amino acid starvation [6] (Fig. 1). Fortunately, the isogeneic strains

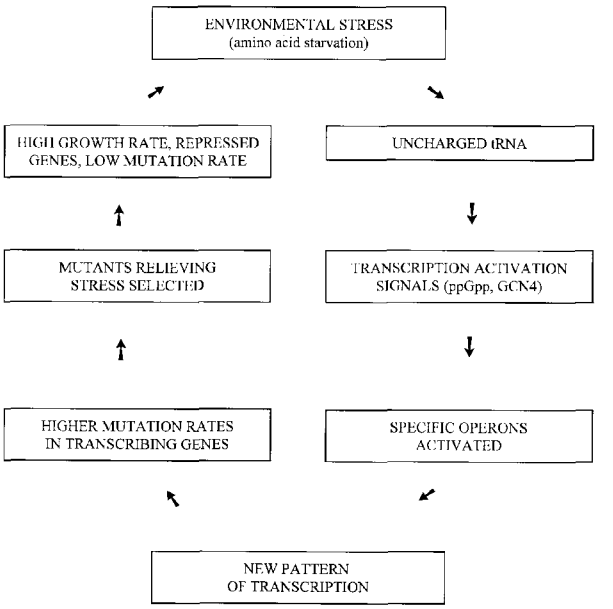

Fig. 2. An algorithm for evolution.

Table 2
A summary of reversion rates in isogenic strains CP78 (*relA*$^+$ *pyrD*$^-$) and CP79 (*relA*$^-$ *pyrD*$^-$)

	CP78 *pyrD*$^-$	CP79 *pyrD*$^-$
Arginine-starved[a]		
A. *argH*$^-$ reversions		
Total cell number $\times 10^8$	1.59 ± 0.47	1.47 ± 0.29
P_0 (No. negative plates/total)	0.15 ± 0.10	0.92 ± 0.02
Mutation rate $\times 10^{-9}$	8.94 ± 0.85	0.43 ± 0.19
B. *leuB*$^-$ reversions		
Total cell number $\times 10^8$	0.92 ± 0.24	0.84 ± 0.11
P_0 (No. negative plates/total)	0.75 ± 0.06	0.97 ± 0.01
Mutation rate $\times 10^{-9}$	2.3 ± 0.70	0.25 ± 0.06
C. *pyrD*$^-$ reversions		
Total cell number $\times 10^8$	0.96 ± 0.28	1.1 ± 0.58
P_0 (No. negative plates/total)	0.62 ± 0.19	0.66 ± 0.11
Mutation rate $\times 10^{-9}$	3.7 ± 1.9	2.8 ± 0.64
Leucine-starved[b]		
A. *leuB*$^-$ reversions		
Total cell number $\times 10^8$	1.86 ± 0.36	1.36 ± 0.37
P_0 (No. negative plates/total)	0.71 ± 0.08	0.92 ± 0.02
Mutation rate $\times 10^{-9}$	1.26 ± 0.26	0.44 ± 0.12
B. *pyrD*$^-$ reversions		
Total cell number $\times 10^8$	1.0 ± 0.16	0.93 ± 0.06
P_0 (No. negative plates/total)	0.83 ± 0.07	0.39 ± 0.07
Mutation rate $\times 10^{-9}$	1.6 ± 0.33	7.3 ± 0.79

[a]Values given are averages of 5–6 mutation rate determinations performed as described previously [31,32]. Cultures were grown in the presence of 0.03 mM arginine and 0.35 mM orotate, washed and plated on minimal medium lacking A: arginine; B: leucine; or C: orotate.

[b]Values given are averages of 5 mutations rate determinations performed as described previously [31,32]. Cultures were grown in the presence of 0.02 mM leucine and 0.35 mM orotate, washed and plated on minimal medium lacking A: leucine; B: orotate.

used in these investigations carry a *malA*$^-$ mutation, and are unable to use maltose as a carbon source. The *relA*$^+$ and *relA*$^-$ strains were starved for a carbon source (glycerol) and plated on minimal medium with maltose as the sole carbon source. Reversion rates were comparable (Table 1), suggesting that ppGpp accumulation was *spoT*- rather than *relA*-mediated under these conditions.

The other control examined the effect of the stringent response on reversion rates of *leuB*$^-$, *argH*$^-$, and of a pyrimidine mutant allele, *pyrD*$^-$, that was transduced into both the *relA*$^+$ and *relA*$^-$ strains. Operons involved in nucleic acid synthesis might be repressed in *relA*$^+$ strains under stringent conditions. As shown in Table 2, starvation of the *relA*$^+$ strain for arginine or leucine resulted in *argH*$^-$ and *leuB*$^-$ reversion rates that were 3–21-fold higher in the *relA*$^+$ than in the *relA*$^-$ strain. In contrast, under the same starvation regimens, *pyrD*$^-$ reversion rates were the same or lower in the *relA*$^+$ compared to the *relA*$^-$ strain. Thus, the effect of the stringent response on mutation rates appears to be specific for amino acid biosynthetic operons. An examination of the nucleotide sequence in the promoter of the *pyrD* gene in *E. coli* revealed GC-rich discriminator sequences [33].

5. Concluding remarks

The examples of growth-dependent, transcription-directed mutations described in this review occurred as a consequence of starvation derepression (the stringent response), genetic derepression [22,23], or substrate induction [20,21,24]. These mutations owed their specificity to differential gene activation. Although their frequency increased in strains deficient in DNA repair [23,34], their specificity was not affected [23]. These mutations do not depend on the mechanisms of homologous recombination [20,34,35] essential to 'adaptive' mutations [34,35] that occur in cells incapable of active growth, DNA synthesis and transcription [36]. Under such circumstances there may be little metabolic potential for responding to specific types of environmental stress; inducing a starving Lac$^-$ strain with IPTG had little effect on the Lac$^+$ mutation rate [37]. Rather, adaptive mutation rate frequencies appear to be primarily controlled by non-specific mechanisms such as the failure of DNA repair systems [34,38]. The major difference between growth-dependent mutations and adaptive mutations could perhaps be summarized as follows: when growing cells are confronted with a change in the environment (e.g. the *lac* inducer or imminent amino acid starvation), they still have the metabolic capabilities for a specific compensatory response, i.e. increased rates of transcription and mutation. However, after 4–5 days with no exogenous energy source cells apparently resort to non-specific increases in all mutation rates in a final effort to produce a mutant that will survive.

Although random mutations will result in more deleterious than potentially advantageous mutants, only the latter will be selected and propagate in a particular environmental niche. Evolution depends upon the availability of variants from which to choose the fittest. An algorithm for evolution is presented in which the environment plays an essential role in directing the kinds of mutations that will occur (Fig. 2). A key prediction inherent in this proposed principle of evolution is that, under any starvation regimen, the extent to which the transcription of a gene increases should be mirrored by an increase in its mutation rate. Mutation rates will of course also be affected by other factors, such as oxidizing agents, UV, the intrinsic mutability of different alleles, the activity of DNA repair enzymes, and variables that could be influenced by starvation conditions, e.g. nucleotide pool levels [39]. The dual role of the environment in (1) derepressing the genes most relevant to a given nutritional stress, resulting in a mutant population enriched with variants of these genes, and (2) selecting the fittest among these variants, should be an ongoing process that is directing and accelerating the rate of microbial evolution.

The algorithm for evolution summarized by Fig. 2 is compatible with Woese's [40] view of macroevolution in bacteria under extreme environmental stress, during which increased mutation rates might be expected to occur and even have a positive selective value. As present-day transcriptional control mechanisms underlie the proposed successive stages of evolution, they would apply to advanced organisms such as *E. coli* and yeast, allowing them to cope with a variety of environmental stresses and to invade new habitats by altering their biochemical capabilities, e.g. becoming resistant to antibiotics, or using a new carbon source [25]. The very early periods of evolution, such as the emergence of basic biosynthetic pathways, must have been subject to quite different environments and control mechanisms, as described by Wächtershäuser [41].

Definitions are essentially arbitrary and subject to criteria such as usefulness and conventionality [42]. In the scenario outlined in this article, mutations would be 'directed' by the environment in that a specific gene or class of genes relevant to the stress imposed would be selected for higher rates of transcription and mutation. The enhanced mutation rates would be specific in so far as the new transcriptional pattern provoked was specific. The mutations, per se, would be random. Words such as 'directs', 'selects', or 'targets' have anthropomorphic overtones. They are simply a convenient, conventional way of speaking; in the context of this review they refer to a prior event or situation (e.g. starvation) that is essential to a subsequent event (e.g. derepression). The term 'adaptive mutation' was not appropriate because of its use in reference to mutations occurring in non-growing cells.

Acknowledgements: For discussions, suggestions, and criticisms of the manuscript I thank H. Paulus, G. Card, K. Tilly, A. Longacre, and W.M. Fitch. Supported in part by The University of Montana and by P.H.S. Grant GM/0D54279 from the National Institutes of Health.

References

[1] Bernhard, R. (1967) Sci. Res. 2, 59–66.
[2] Jablonski, D., Sepkoski, Jr., J., Bottjer, D.J. and Sheehan, P.M. (1983) Science 222, 1112–1124.
[3] Hickey, L.J., West, R.M., Dawson, M.R. and Choi, D.K. (1983) Science 221, 1153–1156.
[4] Magasanik, B. and Neidhardt, F.C. (1987) in: *Escherichia coli* and *Salmonella typhimurium* (Neidhardt, F.C., Ingraham, J.L., Low, K.B., Magasanik, B., Schaechter, M. and Umbarger, H., Eds.) Vol. 2, pp. 1318–1325, American Society of Microbiology, Washington, DC.
[5] Rosenberg, H., Gerdes, R. and Harold, F.M. (1979) Biochem. J. 178, 133–137.
[6] Cashel, M.,Gentry, D.R., Hernandez, V.J. and Vinella, D. (1996) in: *Escherichia coli* and *Salmonella typhimurium* (Neidhardt, F.C., Curtis III, R., Ingraham, J.L., Lin, E.C.C., Low, K.B., Magasanik, B., Reznikoff, W.S., Riley, M., Schaechter, M. and Umbarger, H., Eds.) 2nd edn. Vol. 1, pp. 1458–1496, American Society of Microbiology, Washington, DC.

[7] Stephens, J.C., Artz, S.W. and Ames, B. (1975) Proc. Natl. Acad. Sci. USA 72, 4389–4393.
[8] Hinnebusch, A.C. (1994) Trends Biochem. Sci. 19, 409–414.
[9] Primakoff, P. and Artz, S.W. (1979) Proc. Natl. Acad. Sci. USA 76, 1726–1730.
[10] Ryals, J., Little, R. and Bremer, H. (1982) J. Bacteriol. 151, 1261–1268.
[11] Yang, H.L., Zubay, G., Urm, E., Reiness, G. and Cashel, M. (1974) Proc. Natl. Acad. Sci. USA 71, 63–67.
[12] Zidwick, M.J., Korshus, J. and Rogers, P. (1984) J. Bacteriol. 159, 647–651.
[13] Donini, P., Santonastaso, V. Roche, J. and Cozzone, A.J. (1978) *Escherichia coli*. Mol. Biol. Rep. 4, 15–29.
[14] Ikemura, T. (1985) Mol. Biol. Evol. 2, 13–34.
[15] Sørensen, M.A., Jensen, K.F. and Pedersen, S. (1994) J. Mol. Biol. 236, 441–454.
[16] Mager, W.H. and DeKruijff, J.J. (1995) Microbiol. Rev. 59, 506–531.
[17] Delforge, J., Messenguy, F. and Wiame, J-M. (1975) Eur. J. Biochem. 57, 231–239.
[18] Singer, B. and Kuśmierek, J.T. (1982) Annu. Rev. Biochem. 52, 655–693.
[19] Lindahl, T. and Nyberg, B. (1974) Biochemistry 13, 3405–3410.
[20] Herman, R.K. and Dworkin, N.B. (1971) J. Bacteriol. 106, 543–550.
[21] Brock, R.D. (1971) Mutation Res. II, 181–186.
[22] Cordaro, J.C. and Balbinder, E. (1967) Bact. Proc. CP 15, 51.
[23] Savić, D.J. and Kanazir, D.T. (1972) Mol. Gen. Genet. 118, 45–50.
[24] Datta, A. and Jinks-Robertson, S. (1995) Science 268, 1616–1619.
[25] Lerner, S.A., Wu, T.T. and Lin, E.C.C. (1964) Science 146, 1313–1315.
[26] Selby, C.P. and Sancar, A. (1994) Microbiol. Rec. 58, 317–329.
[27] Hanawalt, P. and Mellon, I. (1993) Curr. Biol. 3, 67–69.
[28] Walker, G.C. (1995) Trends Biochem. Sci. 20, 416–420.
[29] Higashitani, N., Higashitani, A. and Horiuchi, K. (1995) J. Bacteriol. P. 177, 3610–3612.
[30] Maizels, N. (1995) Cell 83, 9–12.
[31] Wright, B.E. (1996) Mol. Microbiol. 19, 213–219.
[32] Wright, B.E. and Minnick, M.F. (1996) Microbiology (in press).
[33] Larsen, J.N. and Jensen, K.F. (1985) Eur. J. Biochem. 151, 59–65.
[34] Longerich, S.L., Galloway, A.M., Harris, R.S., Wong, C. and Rosenberg, S.M. (1995) Proc. Natl. Acad. Sci. USA 92, 12017–12020.
[35] Foster, P.L. (1992) J. Bacteriol. 174, 1711–1715.
[36] Ryan, F.J., Nakada, D. and Schneider, M.J. (1961) Z. Vererbungsl. 92, 38–41.
[37] Davis, B.D., (1989) Proc. Natl. Acad. Sci. USA 86, 5005–5009.
[38] Feng, G., Tsui, H.-C.T. and Winkler, M.E. (1996) J. Bacteriol. 178, 2388–2396.
[39] Kunz, B.A. and Kohalmi, S.E. (1991) Annu. Rev. Genet. 25, 339–359.
[40] Woese, C. (1987) Microbiol. Rev. 51, 221–271.
[41] Wächtershäuser, G. (1988) Microbiol. Rev. 52, 452–484.
[42] Korzybski, A. (1958) Science and Sanity, 4th edn., The Colonial Press, Clinton, MA.