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EXCISION REPAIR GENES OF *SACCHAROMYCES CEREVISIAE*

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Summary. - *In the yeast* Saccharomyces cerevisiae, *at least ten genes are involved in excision repair of DNA damaged by UV radiation and by other agents that distort the DNA helix. Mutations in the* RAD1, RAD2, RAD3, RAD4 *and* RAD10 *genes render cells highly defective in the incision of damaged DNA, whereas mutations in the* RAD7, RAD14, RAD16, RAD23 *and* MMS19 *genes reduce the level of damage excision. This review summarizes the evidence for the involvement of these genes in excision repair and highlights the important features in the structures of the proteins encoded by the various* RAD *genes. The RAD3 protein has been purified and characterized in our laboratory, and it possesses single stranded DNA dependent ATPase and DNA helicase activities. The RAD3 helicase moves along the single-stranded DNA in the 5' → 3' direction. We suggest that this activity plays a role in strand displacement synthesis during excision repair and in DNA replication.*

Riassunto (I geni della riparazione del DNA per escissione in *Saccharomyces cerevisiae*). - *Almeno dieci geni sono coinvolti nel processo di riparazione per escissione del DNA danneggiato dalla radiazione UV e da altri agenti che distorcono l'elica del DNA. Le mutazioni nei geni* RAD1, RAD2, RAD3, RAD4 *e* RAD10 *rendono le cellule altamente difettive nell'incisione del DNA danneggiato, mentre le mutazioni nei geni* RAD7, RAD14, RAD16, RAD23 *e* MMS19 *riducono il livello di escissione del danno. Questa rassegna riassume le evidenze di coinvolgimento di questi geni nella riparazione per escissione e sottolinea le caratteristiche importanti nella struttura delle proteine codificate dai vari geni* RAD. *La proteina RAD3, purificata e caratterizzata nel nostro laboratorio, possiede un'attività ATPasica dipendente da DNA a singolo filamento e un'attività elicasica. La elicasi RAD3 si muove lungo il DNA a singolo filamento in direzione 5' → 3'. Suggeriamo che questa attività ha un ruolo nella sintesi durante il processo di "strand displacement" della riparazione per escissione e durante la replicazione del DNA.*

Introduction

All organisms possess mechanisms that enable them to repair a wide variety of lesions induced in DNA by radiation or chemical DNA damaging agents. Some repair pathways, such as photoreactivation, which acts only on ultraviolet light (UV)-induced pyrimidine dimers, show specificity for the type of lesion. Other repair pathways, such as nucleotide excision repair, which acts on UV-induced pyrimidine dimers as well as bulky DNA adducts, have a broader substrate specificity. In nucleotide excision repair, DNA is incised at or near the damage site, followed by excision of the lesion and DNA synthesis using the opposite intact strand as a template. The detailed mechanism of incision has been elucidated in bacteriophage T4 [1], *Micrococcus luteus (M. luteus)* [2], and *Escherichia coli (E. coli)* [3, 4]. A single polypeptide of 16 kDa in bacteriophage T4 endonuclease V, a glycosylase-apyrimidinic/apurinic endonuclease with specificity for pyrimidine dimers in DNA [5, 6], and of 18 kDa in *M. luteus* [2, 7], incise DNA containing pyrimidine dimers. Both enzymes contain two activities and mediate incision in a two-step process. The first step involves the action of the pyrimidine dimer DNA N-glycosylase acting between the 5' pyrimidine of the dimer and its sugar, while the second step occurs via the apyrimidinic/apurinic (AP) endonuclease activity that breaks the phosphodiester bond on the 3' side of the AP site. Excision of nucleotides followed by repair synthesis using the opposite intact strand as a template and finally ligation to seal the nick comprise the subsequent steps of excision repair.

In *E. coli*, excision repair occurs through the action of a protein complex consisting of three enzymes, UvrA: M_r, 103,874; UvrB: M_r, 76,118; and UvrC: M_r, 66,038 [8]. Pyrimidine dimer-containing DNA is cleaved by the UvrABC excision nuclease at the 8th phosphodiester bond 5' and at the 4th or 5th phosphodiester bond 3' to the dimer [3]. UvrD and polI are required for turnover of the UvrABC complex [9, 10]. UvrA protein has DNA-independent ATPase activity,

and it binds UV-irradiated DNA in the presence of ATP with greater affinity than unirradiated DNA [11]. UvrB protein does not bind UV damaged DNA by itself, but it greatly enhances the ability of the UvrA protein to bind to UV irradiated DNA [4, 12]. The UvrAB protein complex, in the presence of ATP, also unwinds duplex DNA [13]; binding of uvrC protein to the UvrA:UvrB:damaged DNA complex results in the endonucleolytic activity [3, 4, 12]. In addition to the difference in the incision mechanism, the ATP requirement of the *E. coli* UvrABC nuclease makes this enzyme different from the T4 or *M. luteus* enzymes, which do not require ATP. Also, the T4 and *M. luteus* enzymes are specific for UV-induced pyrimidine dimers, whereas the UvrABC nuclease acts on a variety of lesions causing helix distortion.

In humans, excision repair of UV damaged DNA is deficient in cells from xeroderma pigmentosum (XP) patients. A total of nine complementation groups for XP are now known [14], suggesting a greater complexity in excision repair in humans than in *E. coli*. Similarly, in the yeast *Saccharomyces cerevisiae (S. cerevisiae)* at least 10 genes are required for excision repair [15, 16]. Part of the complexity of excision repair in eukaryotic organisms may result from the highly organized nature of eukaryotic chromosomes. Although pyrimidine dimers seem to be formed randomly throughout the genome of irradiated human cells in culture [17], it appears that not all pyrimidine dimers are equally accessible to repair enzymes [18]. Exogenously supplied *M. luteus* UV glycosylase/endonuclease does not recognize a large proportion of pyrimidine dimers in human fibroblasts [19]. Experiments with freeze-thawed permeabilized V79 Chinese hamster or human cells in which T4 UV endonuclease had been introduced, can be interpreted simply if protein bound to DNA makes about 50% of the pyrimidine dimers in DNA inaccessible to repair enzymes. In Chinese hamster ovary cells, over 60% of the UV induced pyrimidine dimers are removed from the active, amplified dihydrofolate reductase *(DHFR)* gene within 26 h following UV irradiation while in the rest of the genome as a whole, only about 15% of the pyrimidine dimers are removed in the same time period [20]. Similar results have been obtained for the human *DHFR* gene, in which more than 60% of pyrimidine dimers are removed within 4 h after UV irradiation whereas only 25% are removed during that time period from the genome overall [21]. In 3T3 fibroblasts, about 85% of the UV induced pyrimidine dimers are removed within 24 h in the transcriptionally active c-*abl* gene while only 22% are removed from the transcriptionally inactive c-*mos* gene [22]. In cultured monkey cells, more rapid repair of pyrimidine dimers occurs in the integrated and transcribed *E. coli gpt* gene than in the genome as a whole [23]. These results suggest that the rate of DNA repair can vary in different genes, depending on their state of expression, changes in chromatin structure and conformation, or other factors.

The yeast *Saccharomyces cerevisiae* has played an important role as a model system in unraveling the various repair mechanisms in eukaryotes. *S. cerevisiae*, is particularly well suited for genetic and molecular studies on DNA repair. It has a well-characterized genetic system and a small genome size, a low DNA content of 1×10^{10} daltons per haploid cell. The recombinant DNA technology available in yeast allows for the isolation of genes, the replacement of the wild type gene in the genome by mutant genes, and overproduction and purification of their products. The availability of a large number of well-characterized repair deficient, radiation sensitive *(rad)* mutants makes *S. cerevisiae* particularly useful for DNA repair studies [24-28]. Since several reviews of DNA repair and mutagenesis in yeast have been published during the past few years [15, 29-32], this review will focus on the *RAD3* group of genes required for nucleotide excision repair of UV damaged DNA. Our goal, and that of other investigators working on DNA nucleotide excision repair in yeast, is to define the biochemical steps involved in this process.

Allelism tests among the *rad* mutants indicated that over 30 different genetic loci exist [33], and survival responses to UV irradiation of double mutant combinations resulted in their classification into three epistasis groups [34-36], which are referred to by a prominent gene in the group. The *rad3* epistasis group consists of mutants which are defective in the excision of UV light induced pyrimidine dimers [37-44]. The *rad6* group contains mutants which show defective postreplication repair of DNA damage induced by UV light [45] as well as reduced mutagenesis following treatment with DNA damaging agents [46-48]. The third group consists of *rad52* and other mutants. The *rad52* mutants are defective in genetic recombination [49-52] and in double strand break repair [53, 54].

Role of *RAD3* epistasis group genes in excision repair

Analyses of the survival responses to UV irradiation of double *rad* mutant combinations revealed epistatic interactions between the *rad1*, *rad2*, *rad3*, and *rad4* mutations [34-36]. Subsequent studies demonstrated that one of the characteristic features of these and other mutants in the *RAD3* epistasis group is enhanced UV mutagenesis compared to *RAD*+ [29]. Since enhanced UV mutagenesis is observed in *E. coli* mutants deficient in nucleotide excision repair, it seemed likely that the yeast *RAD3* group of genes might also be required for nucleotide excision repair. Subsequently, it was shown that *RAD1* [37, 39, 40], *RAD2* [38], *RAD3* [41], *RAD4* [41, 43], *RAD7* [16, 44], *RAD10* [42], *RAD14* [44], *RAD16* [42], *RAD23* [16], and *MMS19* [44] are involved in the removal of pyrimidine dimers from DNA. Mutants of the *RAD1*, *RAD2*, *RAD3*, *RAD4*,

and *RAD10* genes are highly UV sensitive, whereas mutants of the *RAD7, RAD14, RAD16, RAD23,* and *MMS19* genes are not as UV sensitive. However, double mutant combinations in the latter group, as, for example, rad7-Δ rad23-Δ or rad7-Δ rad14-1, show synergism or additivity for UV sensitivity relative to each single mutant [44, 55]. Although most of the excision defective *rad* mutants are not sensitive to MMS, an allele of *rad1* and an allele of *rad4* were obtained among the MMS-sensitive mutants [15]. The mutants in the *RAD3* epistasis group are also generally not sensitive to X-ray irradiation [15]. However, they show cross-sensitivity to DNA damaging agents which produce bulky adducts or distortions in DNA, such as nitrogen mustard [32], nitroquinolone oxide, and DNA crosslinking agents such as 4, 5', 8-trimethyl psoralen + 360 nm light, referred in this article as psoralen + light [56, 57].

Pyrimidine dimer removal

The response of mutants in the *RAD3* epistasis group to photoreactivation following dark holding [58], as well as the enhanced UV mutagenesis suggested that these mutants are defective in pyrimidine dimer removal. Early studies of excision of pyrimidine dimers from yeast DNA utilized relatively high UV fluences that resulted in less than 1% survival. Direct chromatographic identification of pyrimidine dimers in the DNA of cells exposed to UV irradiation revealed that normal *(RAD+)* cells could excise pyrimidine dimers while the *rad1-1* [37] and *rad1-2* [40] mutants could not. The *rad2-17* mutant was also shown to be defective in pyrimidine dimer excision by utilizing an indirect assay which depended on the ability of crude extracts obtained from UV irradiated yeast cells to compete with transforming DNA from UV irradiated *Haemophilus influenzae* for photoreactivating enzyme [38]. A more sensitive assay for pyrimidine dimers utilized the susceptibility of DNA, obtained from yeast cells at different times following UV irradiation, to nicking by T4 endonuclease V [40]. By this method, enzyme-sensitive sites in DNA could be detected with a UV fluence resulting in 12% survival in the highly UV sensitive *rad1-2* mutant [40]. It was shown that pyrimidine dimers remained in the nuclear DNA of the *rad1-2* mutant whereas they were efficiently removed from the repair-proficient *RAD+* strain. On the other hand, pyrimidine dimers induced in mitochondrial DNA were not removed either in the *RAD+* or the *rad1-2* strain [40], indicating that the excision-repair mechanism operates on the nuclear but not on the mitochondrial DNA in yeast. Lack of excision of pyrimidine dimers from mitochondrial DNA of yeast was also demonstrated by Waters and Moustacchi [59]. Other eukaryotes, such as mouse and human, also lack excision repair mechanisms for mitochondrial DNA [60]. The susceptibility of DNA containing pyrimidine dimers to nicking by pyrimidine dimer-specific enzyme from T4 or *M. luteus*, was also used to demonstrate that the *rad3-2* [41], *rad4-3* [43], *rad4-4* [41], *rad7-Δ* [16, 44], *rad10-2* [42], *rad14-1* [44], *rad16-1* [42], *rad23-Δ* [16], and *mms19-1* [44] mutants are defective in pyrimidine dimer removal.

Incision *vs* excision

While the methods described above identified a defect in the removal of pyrimidine dimers, they could not distinguish whether the mutants had a defect in an initial incision step or in a subsequent step, since in the absence of a postincision step, DNA ligase may close the incision nick, resulting in retention of pyrimidine dimers in DNA. Only a few incision breaks are observed in DNA obtained from UV irradiated *RAD+* cells, and these breaks are absent in the *rad1, rad2, rad3,* and *rad4* mutants [61], suggesting an incision defect in these mutants. To distinguish between a defect in the incision step from a defect in a subsequent step(s) of excision repair, Wilcox and Prakash [62] coupled the excision defective *rad* mutations to the *cdc9* mutation, which results in temperature sensitive growth and a thermolabile DNA ligase activity [63]. In a *radx cdc9* mutant, if the *radx* mutant is defective in the initial incision step of excision repair, then the *radx cdc9* mutant would not accumulate single-strand breaks in DNA at the restrictive temperature. On the other hand, if the *radx* mutation allows the initial incision step but is defective in the subsequent step of excision or repair synthesis, then single-strand DNA breaks would accumulate in the *radx cdc9* mutant. Single-strand breaks in DNA after UV irradiation of various *rad cdc9* mutants were monitored by alkaline sucrose sedimentation [62], and the results revealed that the *rad1-1, rad2-5, rad3-2, rad4-4,* and *rad10-2* mutants are defective in making incisions in UV irradiated DNA whereas in the *rad14-1 cdc9-2* and *rad16-1 cdc9-2* mutants, incision breaks were detected. About 30% and 60% as many incision breaks occurred in the *rad14 cdc9, rad16 cdc9* mutants, respectively, as in *RAD+ cdc9*, suggesting that the *rad14* and *rad16* mutants possess significant residual incision capacity.

The ability to remove both crosslinks and monoadducts induced by psoralen + light has also been examined in the mutants defective in pyrimidine dimer removal [57]. Little or no nicking of crosslinked DNA occurs in strains carrying mutations of the *RAD1, RAD2, RAD3, RAD4, RAD10,* and *MMS19* genes [57]. The *rad14-1* [57], *rad7-Δ,* and *rad23-Δ* [16] mutants show significant nicking of crosslinked DNA, but still much less than that observed in the *RAD+* strain. The *rad16-1* mutant is as proficient as the *RAD+* strain in nicking of crosslinked DNA [57]. The *rad cdc9* double mutant combinations were also utilized to determine the effect of these *rad* genes on incision of monoadducts in DNA induced

by psoralen plus near UV light treatment. The *rad1-2*, *rad2-5*, and *rad4-4* mutants were defective in producing incisions at monoadducts as well, whereas the *rad3-2* mutant, defective in incising crosslinked DNA, was proficient in incision of DNA containing monoadducts [64]. Overall, these observations indicate that the *RAD1*, *RAD2*, *RAD3*, *RAD4*, *RAD10*, *MMS19*, and *RAD7*, *RAD14*, *RAD16*, and *RAD23* genes are involved in the removal of DNA damage that distorts the DNA helix.

In summary, mutations in the *RAD1*, *RAD2*, *RAD3*, *RAD4*, *RAD10*, and *MMS19* genes result in a high level of incision defectiveness, and most of these genes are likely to be directly involved in incision. The other group consists of mutants of the *RAD7*, *RAD14*, *RAD16*, and *RAD23* genes which show some degree of incision defect. Some of these genes could encode proteins which affect the accessibility of chromatin to repair enzymes.

In vitro assays for nucleotide excision repair

Attempts to develop cell-free systems for measuring nucleotide excision repair in yeast have met with relatively little success. Lysates from *RAD+* cells, as well as from *rad1-19*, *rad2-2*, *rad3-1*, *rad4-3*, *rad7-1*, *rad10-1*, *rad14-1*, and *rad16-1* mutants, have been shown to remove pyrimidine dimers from UV irradiated DNA that has been specifically nicked with *M. luteus* UV glycosylase [65]. However, specific incision of pyrimidine dimer-containing DNA could not be detected. Pyrimidine dimer-incising activity in cell-free extracts of *RAD+* strains, as well as from *rad1*, *rad2*, *rad3*, *rad4*, *rad10*, and *rad16* mutants was reported by Bekker *et al.* [66, 67], but these results have not been successfully reproduced by other investigators [32]. In human cells, a cell-free system for demonstrating nucleotide excision repair has been described recently [68].

Isolation and characterization of the yeast excision repair genes

Various genes involved in excision repair in yeast have been cloned by complementation of the corresponding *rad* mutation for UV resistance, and their nucleotide sequences determined. The RAD3 protein has been overproduced and purified from yeast cells and some of its biochemical properties characterized. A summary of the cloned *RAD* genes and their encoded proteins is given in Table 1.

The RAD1 *gene.* - The *RAD1* gene was cloned by complementation of the UV sensitivity of a *rad1* mutant by transformation with a yeast genomic library in the 2μ plasmid YEp24 [69, 70]. The *RAD1* gene encodes a transcript of 3.1 kb [69]. The nucleotide sequence of the *RAD1* gene shows an open reading frame of 3,300 nucleotides, which encodes a protein of 1,100 amino acids, with a predicted molecular weight of 126,360 [71]. RAD1 protein contains 15.8% acidic, 14.7% basic, 30.9% hydrophilic, and 38.6% hydrophobic residues (Table 2). As shown in Fig. 1, both amino and carboxyl termini of RAD1 protein are acidic: 27 acidic and 8 basic residues occur in the amino terminal 110 residues while 19 acidic and 7 basic residues occur in the carboxyl terminal 60 residues [71]. The 95 amino acids located between residues 595 and 689 consist of 32 acidic and 10 basic residues, while between residues 516 and 576, basic amino acids are concentrated, there being 18 basic and 6 acidic residues.

Since a partial *RAD1* deletion resulting in loss of the transcriptional and translational signals as well as the first 12 codons of the *RAD1* gene shows intermediate

Table 1. - *Size of proteins predicted from open reading frames (ORF) in the cloned* RAD *genes*

Gene	Number of amino acids in ORF	Predicted protein size	References
RAD1	1100	126,360	71
RAD2	1031	117,847	75
RAD3	778	89,779	78, 84
RAD4	754	87,173	*
RAD7	565	63,705	55
RAD10	210	24,310	98

* R.D. Gietz & S. Prakash, unpublished results

Table 2. - *Amino acid composition of RAD1 protein*

	Number of residues	Percent
Ala	46	4.2
Arg	53	4.8
Asn	78	7.1
Asp	78	7.1
Cys	10	0.9
Gln	45	4.1
Glu	97	8.8
Gly	37	3.4
His	24	2.2
Ile	73	6.6
Leu	126	11.4
Lys	85	7.7
Met	18	1.6
Phe	40	3.6
Pro	40	3.6
Ser	88	8.0
Thr	54	4.9
Trp	12	1.1
Tyr	29	2.6
Val	67	6.1

level of complementation of *rad1-1*, *rad1-19*, and *rad1-Δ* strains [71], we examined whether translation of *RAD1* mRNA begins from the ATG codon at position +1 or from another ATG codon at position +334 in the *RAD1* ORF [71]. The two ATG codons were each changed to ATC codons by site-directed oligonucleotide mutagenesis and the UV sensitivity of mutants examined. The UV resistance of the *rad1-Δ* strain carrying a single copy plasmid with the +334 ATG codon changed to ATC was similar to that of the *RAD+* strain. The UV resistance of the *rad1-Δ* strain carrying a single copy plasmid containing the +1 ATG changed to ATC, or carrying a plasmid with both the +1 and +334 ATGs changed to ATC codons, was similar and intermediate between that of the *RAD+* and *rad1-Δ* strains. Since mutation of the second in-frame ATG has no significant effect on survival after UV irradiation, whereas mutation of the first ATG at position +1 does affect UV survival, translation of the *RAD1* gene very likely initiates from the AUG codon at position +1 in the *RAD1* mRNA.

The RAD2 *gene.* - The *RAD2* gene was cloned in a similar manner as the *RAD1* gene [72, 73] and it encodes a transcript of 3.3 kb [72, 74]. The *RAD2* mRNAs contain heterogeneous 5' ends, mapping at positions -5, -12, -15, -26, -28, -41, -47, and -62 [75], where +1 refers to the first base of the translation initiating ATG codon. The *RAD2* open reading frame encodes a protein of 1,031 amino acids with a predicted molecular weight of 117,847 [75]. RAD2 protein contains 17.8% acidic, 15.3% basic, 31.6% hydrophilic, and 35.3% hydrophobic residues (Table 3). The carboxyl terminus of RAD2 protein is highly basic: 18 basic and 3 acidic residues occur in the last 44 amino acids (Fig. 2). Deletion of the 78 carboxyl terminal amino acids from RAD2 protein, which include 23 basic and 9 acidic residues, results in complete loss of RAD2 function [75].

The *RAD2* gene is induced upon treatment of cells with DNA damaging agents [75, 76]. A 9-fold and 5-fold increase in *RAD2* mRNA levels was observed after exposure of yeast cells to 25 J/m^2 and 50 J/m^2 UV light, respectively [75]. Yeast cells containing an integrated *RAD2-lacZ* fusion exhibit a 4- to 6-fold increase in β-galactosidase expression following treatment with UV light, γ-radiation, 4-nitroquinoline-1-oxide, or nalidixic acid [76]. Induction of other genes in the *RAD3* epistasis group has not been observed [77, 78].

The RAD3 *gene and protein.* - The *RAD3* gene was also cloned by complementation of the UV sensitivity of a *rad3* mutant using a yeast genomic library in the 2μ vector YEp24 [79, 80]. *RAD3* is the only gene that is required for incision of damaged DNA and is also essential for cell viability [80, 81], indicating that *RAD3* plays an important role in other cellular processes in addition to incision of damaged DNA. In contrast, none of the *E. coli Uvr* genes involved in incision are essential for viability. The role of *RAD3* in the maintenance of cell viability remains unknown [82, 83].

The RAD3 protein is 778 amino acids long [78, 84] with a calculated molecular weight of 89,779, and contains 40.7% nonpolar, 29.4% polar, 15.3% acidic, and 14.5% basic amino acids (Table 4). The distribution of acidic and basic residues in RAD3 protein is shown in Fig. 3. A concentration of acidic residues occurs in the carboxyl terminal portion of the protein; in the last 20 amino acids, there are 12 acidic residues and only 1 basic residue. Seven of the acidic residues, located between amino acids 768 to 774, are present in tandem.

Table 3. - *Amino acid composition of RAD2 protein*

	Number of residues	Percent
Ala	49	4.8
Arg	51	5.0
Asp	83	8.1
Asn	66	6.4
Cys	4	0.4
Gln	44	4.3
Glu	100	9.7
Gly	51	5.0
His	10	1.0
Ile	56	5.4
Leu	84	8.1
Lys	97	9.4
Met	25	2.4
Phe	49	4.7
Pro	41	4.0
Ser	86	8.3
Thr	51	5.0
Trp	10	1.0
Tyr	23	2.2
Val	51	5.0

Table 4. - *Amino acid composition of RAD3 protein*

	Number of residues	Percent
Ala	38	4.9
Arg	49	6.3
Asn	27	3.5
Asp	48	6.2
Cys	13	1.7
Gln	27	3.5
Glu	71	9.1
Gly	33	4.2
His	14	1.8
Ile	57	7.3
Leu	81	10.4
Lys	50	6.4
Met	26	3.3
Phe	33	4.2
Pro	32	4.1
Ser	54	6.9
Thr	42	5.4
Trp	4	0.5
Tyr	33	4.2
Val	46	5.9

A *RAD3* DNA fragment in which the carboxyl terminal 25 amino acid codons are replaced by a pBR322 encoded sequence of 17 amino acids exhibits full repair and viability activity [84]. The replacement of the normally acidic carboxyl terminus of RAD3 protein with pBR322 sequences results in a slightly basic carboxyl terminal region [84].

Between amino acids 45 and 49, the RAD3 protein contains the conserved sequence **GlyX-Gly-Lys-Thr** present in various proteins that bind and hydrolyze ATP or other nucleotides [85]. The presence of this sequence in the RAD3 protein suggested that it may bind and hydrolyze ATP. Dr Patrick Sung in our laboratory has purified the RAD3 protein and characterized its biochemical activities. RAD3 was purified to near homogeneity from yeast strains carrying a RAD3 overproducing plasmid. The RAD3 protein catalyzes the hydrolysis of ATP to ADP and P_i in the presence of a single-strand DNA cofactor [86]. No ATP hydrolysis is observed in the absence of DNA, or in the presence of UV irradiated or unirradiated double stranded DNA. Stimulation of the ATPase activity is not observed with UV irradiated single-stranded DNA. The ATPase activity requires Mg^{+2}, but Mn^{+2} can substitute for Mg^{+2}. The pH optimum for ATP hydrolysis is near 5.6. The RAD3 ATPase activity is inhibited by anti-RAD3 antiserum. The RAD3 protein also possesses a DNA helicase activity that unwinds duplex regions in DNA substrates that were constructed by annealing DNA fragments of 71-851 nucleotides to circular, single-stranded M13 DNA [87]. The DNA helicase activity is dependent on ATP hydrolysis, also has a pH optimum near 5.6, and is inhibited by anti-RAD3 antibodies. The RAD3 helicase translocates along single-stranded DNA in the 5' → 3' direction. Like RAD3, the DNA helicases known to be involved in DNA replication, such as phage T7 gene 4, phage T4 gene 41, and *E. coli* DnaB also move along single-stranded DNA in the 5' → 3' direction [88-90], whereas UvrD helicase of *E. coli*, involved in excision and mistmatch repair [8], moves unidirectionally in the 3' → 5' direction [91]. RAD3 helicase activity could be required for strand displacement synthesis in excision repair [87] and it might play a role in DNA replication.

The RAD4 *gene.* - It was not possible to clone the *RAD4* gene by complementation of the UV sensitivity of *rad4* mutants with a yeast genomic library [92]. Fleer *et al.* [92] have shown that due to toxic effects of RAD4 protein, plasmids carrying a wild type *RAD4* gene cannot be propagated in *E. coli*, and only a mutationally inactivated *rad4* gene is recovered from *E. coli*. The *RAD4* gene is tightly linked to the *SPT2* locus on chromosome V [93]. An *ARS1, URA3* containing plasmid, pR140 and its integrating derivative pR169, carry an *spt2-1* allele and a mutant *rad4* gene [92]. Fleer *et al.* [92] cloned the wild type *RAD4* gene by gap repair of the *rad4* sequence, present in plasmid carrying the *spt2-1* gene, by gene conversion from information provided by the genomic *RAD4* gene. Reversible inactivation of the wild type *RAD4* gene, as by insertion of a restriction fragment within it, permits propagation of *RAD4* plasmid in *E. coli* [92]. The *RAD4* gene is about 2.3 kb in size, is transcribed in the same direction as the *SPT2* gene, and lies immediately upstream of the *SPT2* gene [92]. The *RAD4* gene has also been isolated in our laboratory and its nucleotide sequence determined. It encodes a protein of 754 amino acids with a molecular weight of 87,173 (R. D. Gietz and S. Prakash, unpublished results).

The RAD7 *gene.* - The *RAD7* gene is tightly linked to *CYC1*, on the right arm of chromosome X, and is part of the COR cluster *(CYC1-OSM1-RAD7)* spanning about 1.5 centimorgans [94]. Another cluster of genes, designated ARC, is located on chromosome V, and includes the *ANP1, RAD23,* and *CYC7* genes [95]. McKnight *et al.* [95] have proposed that these two gene clusters are related by duplication and transposition. The 1.8 kb *RAD7* mRNA encodes a protein of 565 amino acids with a predicted size of 63.7 kDa [55]. Multiple transcription initiation sites occur in *RAD7* and are located between positions -61 and -8 relative to the +1 translation initiating ATG codon [55]. The RAD7 protein has a high leucine content, and it contains 37.3% nonpolar, 34.0% polar, 14.9% acidic, and 13.8% basic amino acids (Table 5). The amino-terminal end of the protein contains the major clusters of both acidic and basic amino acids, where the first 200 amino acids contain 49% of the total charged residues (Fig. 4). The first 10 amino acids contains a cluster of 5 basic residues followed by a predominantly acidic region that spans about 120 residues. Seven contiguous arginine

Table 5. - *Amino acid composition of RAD7 protein*

	Number of residues	Percent
Ala	27	4.8
Arg	33	5.8
Asn	38	6.7
Asp	38	6.7
Cys	11	1.9
Gln	20	3.5
Glu	46	8.1
Gly	27	4.8
His	7	1.2
Ile	36	6.4
Leu	77	13.6
Lys	38	6.7
Met	8	1.4
Phe	21	3.7
Pro	14	2.5
Ser	53	9.4
Thr	32	5.7
Trp	3	0.5
Tyr	11	1.9
Val	25	4.4

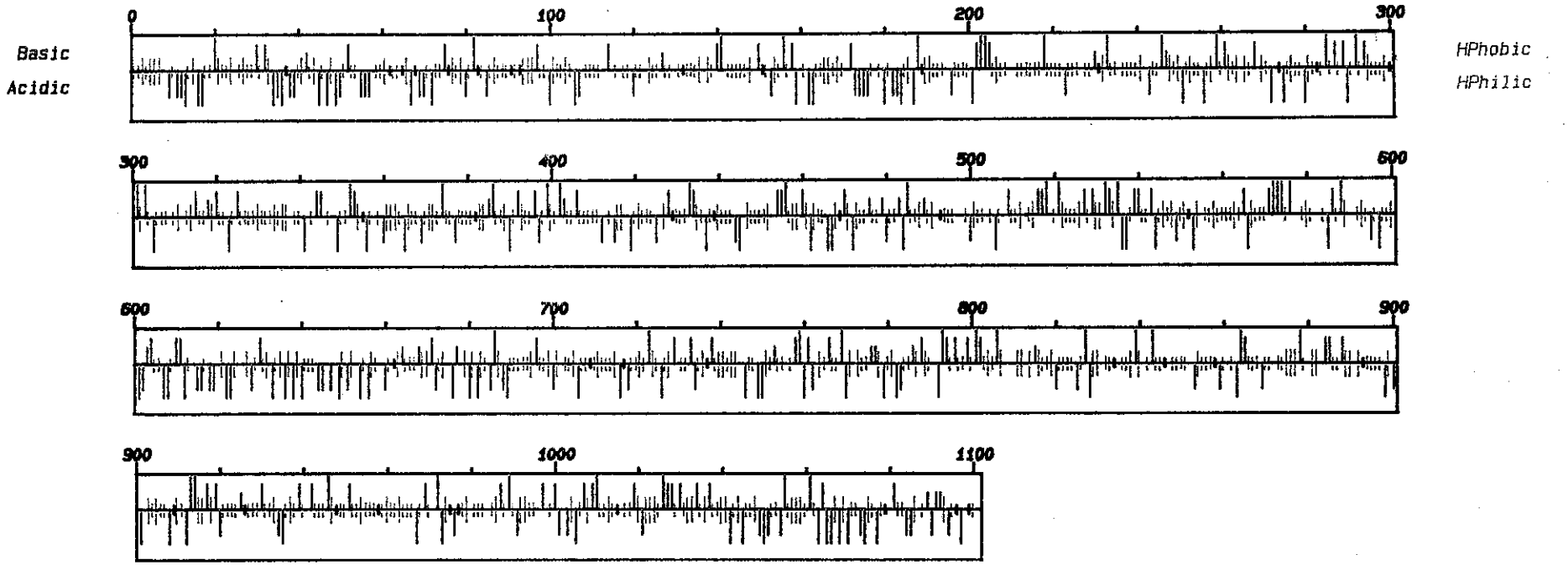

Fig. 1. - Charge profile of the RAD1 protein. Red vertical lines extending above the horizontal line represent the basic amino acids, while those extending below the horizontal line represent the acidic residues. Short black lines that cross the horizontal represent proline residues. Green vertical lines represent hydrophobic residues while blue horizontal lines represent hydrophilic residues.

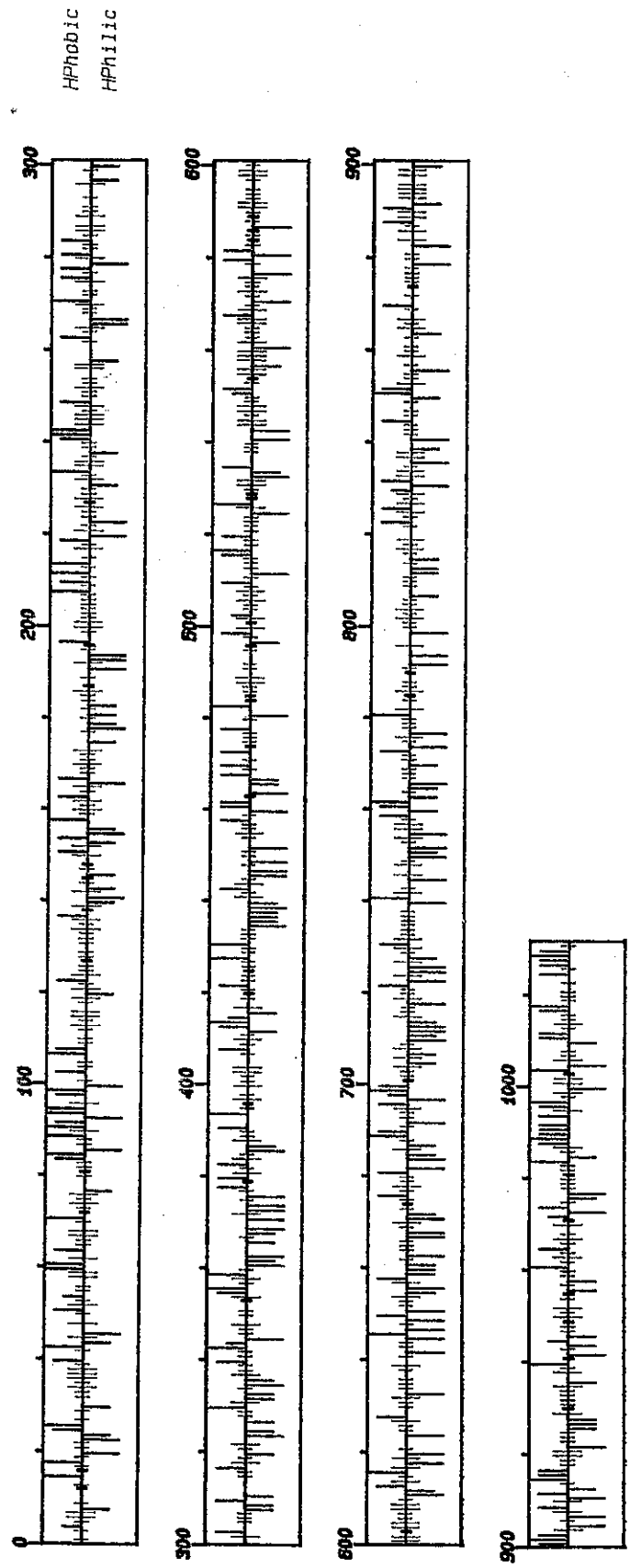

Fig. 2. - Charge profile of the RAD2 protein. Red vertical lines extending above the horizontal line represent the basic amino acids, while those extending below the horizontal line represent the acidic residues. Short black lines that cross the horizontal represent proline residues. Green vertical lines represent hydrophobic residues while blue horizontal lines represent hydrophilic residues.

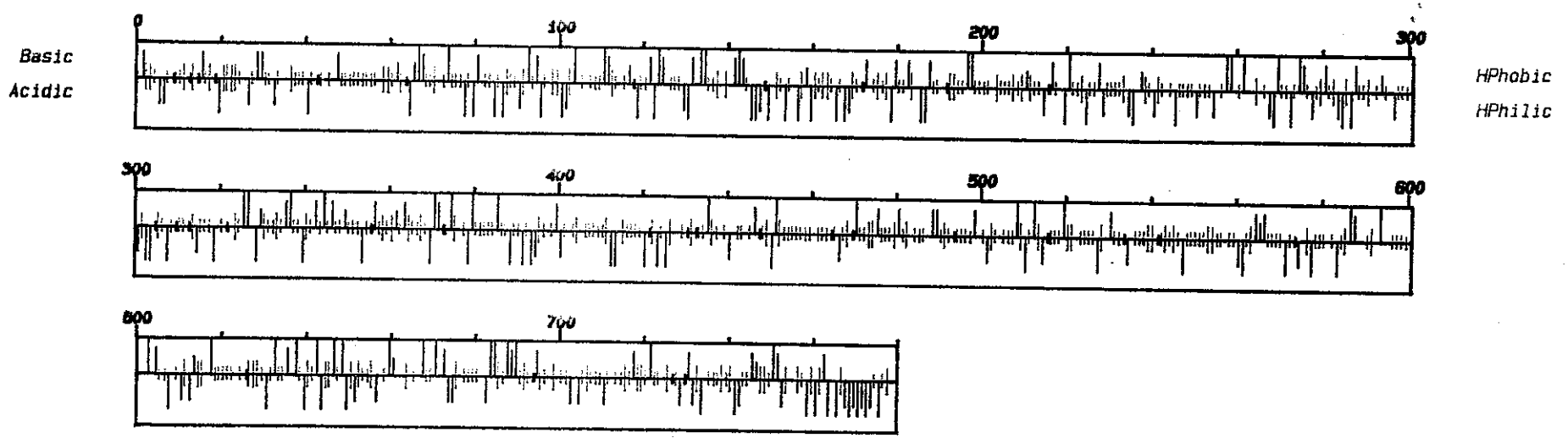

Fig. 3. - Charge profile of the RAD3 protein. Red vertical lines extending above the horizontal line represent the basic amino acids, while those extending below the horizontal line represent the acidic residues. Short black lines that cross the horizontal represent proline residues. Green vertical lines represent hydrophobic residues while blue horizontal lines represent hydrophilic residues.

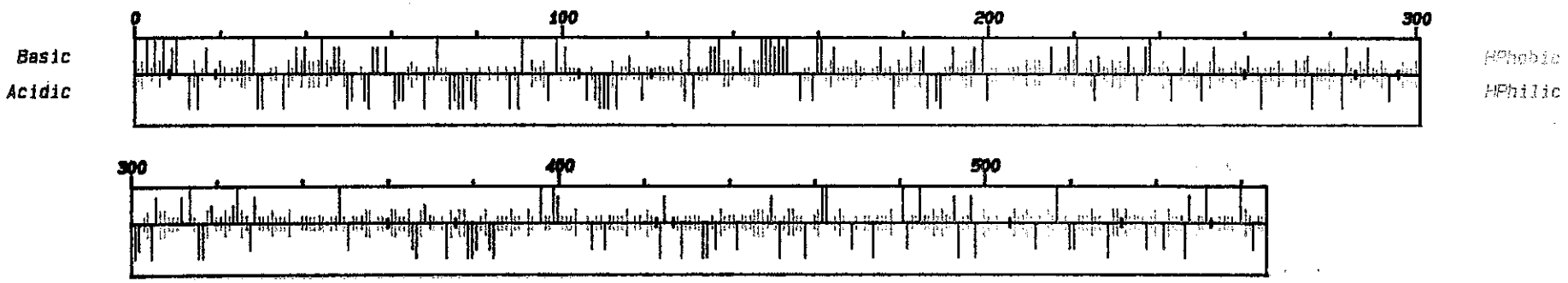

Fig. 4. - Charge profile of the RAD7 protein. Red vertical lines extending above the horizontal line represent the basic amino acids, while those extending below the horizontal line represent the acidic residues. Short black lines that cross the horizontal represent proline residues. Green vertical lines represent hydrophobic residues while blue horizontal lines represent hydrophilic residues.

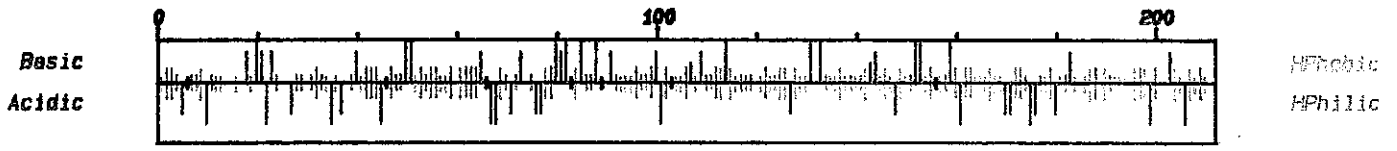

Fig. 5. - Charge profile of the RAD10 protein. Red vertical lines extending above the horizontal line represent the basic amino acids, while those extending below the horizontal line represent the acidic residues. Short black lines that cross the horizontal represent proline residues. Green vertical lines represent hydrophobic residues while blue horizontal lines represent hydrophilic residues.

and lysine residues are found between amino acids 147 and 153. About the same number of acidic and basic residues are found in the central portion of RAD7 protein while the carboxyl terminal 200 residues contain about twice as many acidic as basic amino acids. The amino terminus of RAD7 is highly hydrophilic whereas the carboxyl terminal region is very hydrophobic [55].

A *RAD7* gene lacking the entire 5' upstream region as well as the first 209 bp of the RAD7 open reading frame and present in a multicopy plasmid, is able to fully complement the *rad7-Δ* mutation [55]. The complementation could have resulted from the presence of high quantities of a partially active RAD7 protein due to the high copy number vector, or to complementation by the genomic RAD23 protein, which, because of its probable functional relationship with RAD7, might provide the function absent in the partially deleted RAD7 protein, or both. UV survival of the *rad7-Δ* strain containing the amino-terminally deleted *RAD7* gene present on a single copy vector is intermediate between that of the *rad7-Δ* mutant and the *RAD+* strain, indicating that copy number is only partly responsible for the effect. In contrast to full complementation of the *rad7-Δ* mutation, the multicopy plasmid carrying the amino terminally deleted *RAD7* gene did not show any complementing ability in a *rad7-Δ rad23-Δ* double mutant. These results suggest that the RAD23 protein can compensate for the function which is absent in the amino terminal deletion of RAD7. However, since the *rad7-Δ* and *rad23-Δ* single mutations are not complemented by multicopy plasmids carrying the *RAD23* and *RAD7* genes, respectively, the *RAD7* and *RAD23* proteins must be functionally distinct [55].

The RAD10 *gene.* - The *RAD10* gene was cloned by complementation [96, 97]. The RAD10 protein predicted from the the nucleotide sequence contains 210 amino acids with a calculated molecular weight of 24,310 [98]. The RAD10 protein contains 35.2% non-polar, 40.5% polar, 11% acidic and 13.3% basic residues, and is thus somewhat basic (Table 6). The middle portion of RAD10 is very basic (Fig. 5): the 82 residues from amino acids 78 to 159 contain 17 basic and only 3 acidic residues [98]. In addition to containing over half of the total basic residues, these 82 amino acids contain 8 of the 10 tyrosine residues of RAD10. This region might be involved in DNA binding through ionic interactions and by intercalation of tyrosine residues between the DNA bases. Intercalation by tyrosine residues has been implicated in the binding of bacteriophage fd gene 5 protein and T4 gene 32 protein to single-stranded DNA [99-101], and it may also be involved in RAD10 binding to DNA.

Table 6. - *Amino acid composition of RAD10 protein*

	Number of residues	Percent
Ala	9	4.3
Arg	13	6.2
Asn	21	10.0
Asp	12	5.7
Cys	1	0.5
Gln	11	5.2
Glu	11	5.2
Gly	7	3.3
His	2	0.9
Ile	12	5.7
Leu	22	10.4
Lys	13	6.2
Met	4	1.9
Phe	9	4.3
Pro	7	3.3
Ser	17	8.1
Thr	18	8.5
Trp	2	0.9
Tyr	10	4.7
Val	9	4.3

Extensive homology occurs between the RAD10 protein and the protein encoded by the human excision repair gene *ERCC-1* [102], suggesting an evolutionary conservation of DNA repair genes in eukaryotes.

Future directions

The availability of the cloned *RAD* genes involved in excision repair makes it feasible to overproduce and purify their encoded proteins from yeast cells, as has already been achieved with RAD3. The purified proteins could then be examined for biochemical activities individually and in various combinations, the incision activity could be reconstituted *in vitro*, and the incision mechanism defined.

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