Environmental Health Perspectives
Vol. 62, pp. 115-117, 1985

Cellular Responses to DNA Damage

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For many years, the study of the regulation of the SOS network was complicated by both the complexities of the responses and the interrelationships of the key regulatory elements. However, recently the application of powerful genetic and molecular biological techniques has allowed us to gain a detailed picture of the regulation of this complex network. The network is now known to consist of more than 17 genes, each of which is repressed by the LexA protein. Induction of the genes in the SOS network occurs when the RecA protein becomes activated in response to a signal generated by DNA damage. Two of the genes in this network, *umuD* and *umuC*, are absolutely required for mutagenesis by UV and various carcinogens. The *umuD* and *umuC* genes have molecular weights of 16,000 and 45,000 daltons, respectively, and are organized in an operon repressed by LexA. The mutagenesis-enhancing plasmid pKM101 carries two genes *mucA* and *mucB*, which are analogs of the *umuD* and *umuC* genes, respectively.

Introduction

E. coli has two major independent regulatory networks that are induced in response to DNA damage: (1) the SOS network, which is controlled by the RecA and LexA proteins (*1,2*), and (2) the adaptive response network, which is controlled by the Ada protein (*2,3*). Between them, these two regulatory systems control the induction of more than 21 genes. The SOS network controls the expression of genes whose products are known to play roles in excision repair, daughter-strand gap repair, double-strand break repair, methyl-directed mismatch repair, and SOS processing and also controls the expression of genes involved in cellular responses to DNA damage. The adaptive response network controls the expression of proteins with roles in the direct removal of methyl and ethyl groups from DNA, in the excision repair of alkylated bases, and perhaps in other repair processes. In addition, studies showing that *E. coli* has an inducible system for repairing oxidizing damage have suggested that there may be at least one other regulatory circuit governing the expression of DNA repair proteins (*4*). Also, a number of DNA-damaging agents induce genes that are members of the heat shock regulatory network (*5*), but to date there is no evidence that any of the heat shock proteins are DNA repair proteins. The manner in which cells are able to respond to challenges to their genetic material is strongly affected by which of these systems has been induced and to what extent.

The SOS system was the first regulatory network to be identified that is induced by DNA damage. It is the largest, most complex, and best understood DNA damage-inducible network to be characterized to date. I will briefly summarize our understanding of the regulation of the SOS system and will focus on the importance of inducible cellular functions in UV and chemical mutagenesis.

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The SOS Responses

Exposure of *E. coli* to agents that damage DNA or interfere with DNA replication results in the induction of a diverse set of physiological responses termed the SOS responses which include: an increased capacity to reactivate UV-irradiated bacteriophage (Weigle-reactivation), a capacity to mutate UV-irradiated bacteriophage (Weigle-mutagenesis), the induction of functions that allow bacteria to be mutated by UV and a variety of agents, filamentous growth, an increased capacity to repair double-stranded breaks, an alleviation of restriction, and a capacity to carry out long patch excision repair (*1,2,6*). The existence of a common regulatory circuit controlling the expression of these responses was first clearly postulated by Defais et al. (*7*), and this hypothesis was amplified and developed by Radman(*8,9*). The earlier genetic and physiological studies of the SOS responses have been reviewed by Witkin (*6*) and more recent developments by Little and Mount (*1*) and Walker (*2*).

A Molecular Description of SOS Regulation

The SOS responses are now known to be due to the induction of more than 17 genes which have often been referred to as *din* (damage-inducible) genes (*2,10*). One approach that has proved to be particularly useful for studying the regulation of the SOS responses in *E. coli* has been to make extensive use of operon fusions, since

they allow the issue of the regulation of SOS genes to be addressed without having to deal experimentally with the complex phenotypes caused by these genes. Many of the fusions that have been isolated were generated *in vivo* by using the Mu *dl* (Ap *lac*) bacteriophage (*11*). We have used the phage both to search for genes that were members of the SOS regulatory network (*10*) and to generate fusions to genes such as *uvrA* (*12*) and *umuDC* (*13*), which we suspected of being part of this network.

The LexA protein apparently serves as the direct repressor of every SOS gene that has been identified to date (*1,2*). In addition, a number of genes have been identified on naturally occurring plasmids which are repressed by the *lexA* protein; the case of the *mucAB* genes of *E. coli* is discussed below. Exposure of cells to DNA-damaging agents (e.g., UV or mitomycin C), or to treatments which interfere with DNA replication (e.g., shifting certain mutants that are temperature-sensitive for DNA replication to the restrictive temperature), generates an inducing signal which activates RecA molecules. When activated RecA interacts with a LexA monomer, an -ala-gly- bond in the LexA molecule is cleaved. As the LexA molecules in a cell are inactivated by this proteolytic cleavage, the various SOS genes are expressed at increased levels, and the SOS responses mediated by the products of these genes are observed. The repressors of bacteriophage such as lambda have homology to LexA (*14*) and are similarly cleaved at an -ala-gly- bond when they interact with activated RecA leading to prophage induction. As DNA repair helps the cells recover from the DNA-damaging treatment, the inducing signal disappears, so that RecA molecules cease to be activated (*15*). LexA molecules then accumulate in the cells and repress the SOS genes.

Cellular Functions Required for UV and Chemical Mutagenesis

In *E. coli*, mutagenesis by UV and various chemicals is not a passive process; rather, it requires the participation of various cellular components. Mutations at four different chromosomal loci—*recA*, *lexA*, *umuC*, and *umuD*— can make cells nonmutable by UV and a variety of chemical agents. The $recA^+$ and $lexA^+$ gene products are involved in the regulation of the set of SOS genes in *E. coli* that are induced by DNA damage so that mutations at these loci are pleiotropic. In contrast, *umuC* and *umuD* mutants are nonmutable with many agents and are deficient in Weigle-reactivation, but still exhibit the rest of the SOS responses (*16–20*). Thus *umuC* and *umuD* are the best candidates for genes whose products are uniquely involved in "error-prone" repair. By using the Mu *dl*(Ap *lac*) bacteriophage to generate an operon fusion to the *umuC* gene we have shown that expression of the *umuC* gene was induced by DNA damage in a *recA lexA*-dependent fashion (*13*).

The plasmid pKM101, which was derived from the clinically isolated plasmid R46 by *in vivo* means (*20*), increases the susceptibility of cells to mutagenesis in a $recA^+lexA^+$-dependent manner (*21*), and is able to suppress the nonmutability and UV sensitivity of *umuC* mutants (*22*). An approximately 2000 base-pair (bp) region of pKM101 termed *muc* has been shown to be required for these effects (*23*) and codes for analogs of the *umuD* and *umuC* gene products (*22,24*).

Cloning the *umuDC* Locus of *E. coli*

We have recently cloned the *umuC* locus of *E. coli* (*18*). We initially attempted to obtain the *umuC* gene by preparing a library of *E. coli* DNA cloned into pBR322 and screening the recombinant plasmids for their ability to complement the nonmutability of a *umuC36* mutant, but this approach proved unsuccessful. Instead, a probe to the *umuC* region was derived from a *umuC*::Tn*5* mutant and was used to screen a lambda library of *E. coli* DNA. The *umuC* locus was then subcloned from a $umuC^+$ bacteriophage onto a low copy number plasmid. Through a combination of subcloning and Tn*1000* mutagenesis, we identified a region of 2.2 kilobases (kb) which contains the information necessary to complement *umuC* mutations. This region of DNA codes for two polypeptides with molecular weights of 16,000 and 45,000 daltons. The genes for these proteins are organized in an operon that is repressed by the LexA protein. Complementation of previously isolated *umuC* mutations revealed that these two proteins correspond to two complementation groups—*umuD* which codes for the 16,000-dalton protein and *umuC* which codes for the 45,000-dalton protein—and that therefore both proteins are essential for "error-prone repair" in *E. coli* (*18*). Shinagawa and Kato have independently cloned the *umuDC* locus, and their results are in agreement with ours (*19*).

Cloning the *mucAB* Locus of pKM101

We have also recently subcloned the *muc* locus of pKM101 and examined its structure in detail (*24*). Like the *umuDC* locus of *E. coli*, the *muc* locus of pKM101 consists of two genes: *mucA*, which codes for a 16,000-dalton protein, and *mucB*, which codes for a 45,000-dalton protein. These two genes are organized in an operon (*24,25*). By constructing a *mucB'-lac'Z* gene fusion, we have been able to show that the *mucAB* operon is induced by DNA damage and is repressed by LexA (*25*). Complementation studies have indicated that both proteins are required for pKM101's effects on mutagenesis (*24*).

The Relationship Between *umuDC* and *mucAB*

To further analyze the relationship between the *umuDC* genes and the *mucAB* genes we have deter-

mined the DNA sequence of each operon. The sequences of the *umu* proteins and the *muc* proteins have undergone considerable evolutionary divergence. The deduced amino acid sequences of the UmuD and MucA proteins are approximately 41% homologous, and those of the UmuC and MucB proteins are approximately 55% homologous. Intriguingly, the UmuD and MucA proteins are about 30% homologous to the carboxy-terminal region of the LexA protein including that portion containing the cleavage site. The significance of this homology, if any, is not yet clear. Possibilities include the UmuD and MucA proteins being proteolytically cleaved in a RecA-mediated fashion, the proteins interacting physically with the RecA protein, or the proteins existing as dimers as LexA does (K. L. Perry, S. J. Elledge, L. Marsh, L. Dodson, L. Vales, and G. C. Walker, unpublished results).

The roles of the UmuD and UmuC gene products in SOS processing are presently unknown. If one were to make the assumption that SOS processing involves the misincorporation of bases across from DNA lesions, then these proteins could influence such a process by such formal possibilities as coding for a new polymerase activity themselves, modifying the properties of an existing polymerase, or regulating the induction of yet another protein which then participates directly in the biochemical mechanism. Alternatively, the UmuD and UmuC proteins could be involved in some step subsequent to the actual misincorporation event.

This work was supported by grant CA21615 from the National Cancer Institute and grant GM28988 from the National Institute of General Medical Sciences.

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