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The program has three interrelated aims. The first stage of work involves recombinant construction of polymerase cDNA into a family of selected expression vectors which in most cases will possess an inducible promoter. During the first year, the cDNA was inserted in both sense and antisense orientations and also site-directed mutants. We were also concerned with construction (I-D) and expression of site-directed mutants and potential inhibitory peptides in vivo in order to eventually modulate the activity of poly(ADP-Rib) polymerase in cells, upon induction during DNA repair. The various expression vectors will be stably transfected into a variety of eukaryotic cells generally by co-transfection with a selectable gene. We expect various levels of overexpression and underexpression of poly(ADP-Rib) polymerase. In the case of the site-directed mutants and the inhibitory peptides (IID), we anticipate cells with reduced capacity for ADP-ribosylation. Biochemical and molecular biolo characterizations of the gene products of the various transfected cells are proposed prior to cytotoxicity or DNA repair analysis. These will include : Southern analysis (OVER)

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to confirm integrated copies of the cDNA; both Northern and primer extension analysis of cellular mRNA to confirm that upon induction actual expression of the foreign gene occurs; immunoprecipitation of poly(ADP-Rib) polymerase in vivo after induction. Finally, using the well characterized cells obtained above a variety of cytotoxicity, mutagenicity DNA repair protocols will be initiated to indicate the effects on recovery of cells from various DNA damaging as occasioned by environmental toxic agents when a requirement for ADP-ribosylation is encountered.

ANNUAL TECHNICAL REPORT 1989

(a). A COMPREHENSIVE LIST OF THE OBJECTIVES OF THE RESEARCH EFFORT AND STATEMENT OF WORK

It has long been recognized that NAD is a major metabolite of the eukaryotic cell nucleus. The enzymes involved in both the synthesis and breakdown of NAD are associated with chromatin within the nucleus. The rate of NAD synthesis in the eukaryotic nuclei is extremely high (10^5 molecules/sec/cell). Approximately 95% of this replaces the NAD that is catabolized in the nucleus (for the poly ADP-ribosylation modification of nuclear proteins) and only 5% maintains they cytoplasmic NAD for growth.

The enzyme poly(ADP-Rib) polymerase requires DNA for activity, and it is significant that the catalytic activity of this enzyme is directly coordinated to the number of DNA strand breaks in DNA, both *in vitro* as well as *in vivo*. Accordingly, significant reductions in cellular NAD levels reflect increased poly ADP-ribosylation, due to DNA breaks, and hence it is of significance to this application that 38 organophosphorus and methyl carbonate insecticides in doses as low as 0.6 parts per million cause lowering of NAD. The levels of NAD were directly correlated with teratogenesis. This data is presented in the next page. It is our opinion that the poly ADP-ribosylation modification of chromatin-associated proteins plays an important function during the repair of DNA strand breaks in cells due to a variety of environmental toxic agents.

Our laboratory, was the first to isolate and clone a full-length cDNA for this enzyme. *We also showed that this cDNA, in an appropriate* vector, can be expressed in eukaryotic cells. This will permit direct experiments, using recombinant DNA techniques to test for the role of this enzyme in DNA repair and recovery from toxic agents. For example, we propose to over-or under-produce the polymerase, inhibitory domains or site-directed mutations of enzymes in cells exposed to environmental toxic agents to assess the effects on repair and cell survival. Accordingly, the use of the molecular techniques as well as the complete amino acid sequence of the enzyme which have been established during the past granting period should allow us to learn considerably more about the mechanism and role of this enzyme in cells exposed to stressful environments.

An initial approach has been during the last year to carefully establish techniques to express, regions, orientation, site-directed and mutations of the polymerase cDNA in animal cells and confirm that these products are targeted to the nucleus.

In AIM I, we initially proposed to extend our preliminary data on the insertion of full-length polymerase cDNA into various inducible and non-inducible expression vectors and retroviral vectors in both sense and antisense orientations. This will allow us to either inhibit (i.e. through antisense mRNA expression) or intensify the translation of polymerase in a variety of eukaryotic cells. A complementary approach was proposed in AIM II where various functional domains of the polymerase as well as site-directed mutants (based upon sequence data obtained, during the earlier granting period) will be constructed into inducible expression vectors to test whether selective inhibitors can be favorably used in cells.

Once it is verified that both the engineered mRNA's and appropriate peptides are expressed in *in vivo*, procedures in AIM III will test for cytotoxicity and DNA repair potential and mutagenesis repair of the various reconstructed cells obtained in AIMS I AND II. It is anticipated that these studies will ultimately contribute new information on the mechanisms of the poly ADP-ribosylation modification and how cells recover from damage caused by specific environmental toxic agents such a polyacromatic hydrocarbon, pesticides etc.

AIM I: Construction of poly(ADP-Rib) polymerase cDNA in sense and antisense orientations into expression vectors with inducible promoters.

A. Choice of vectors and cloning strategies.

B. Stable Transfection into various eukaryotic cells; Quantitation of mRNA and expressed proteins after induction.

C. Effects of induced constructions on endogenous polymerase synthesis.

AIM II: Expression in cells of poly(ADP-Rib) polymerase functional domain peptides and site-directed mutants.

A. Cloning strategies and biochemical verifications of expressed peptides driven by various inducible promoters.

B. Effects on cellular poly ADP-ribosylation and cell viability.

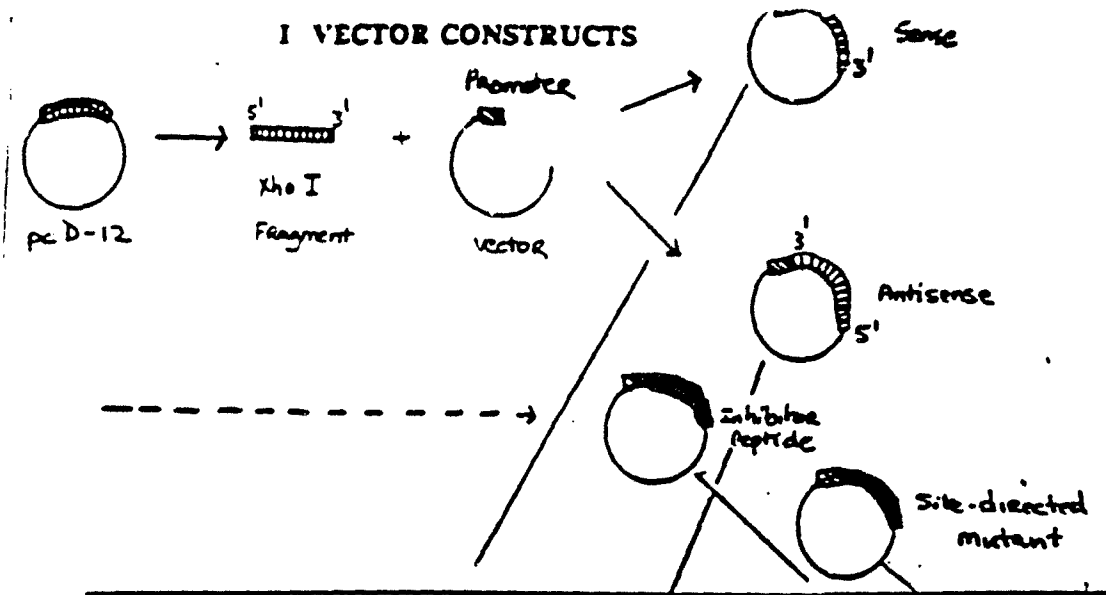
AIM III: Cytotoxicity and DNA repair studies with environmental toxic agents.

GENERAL OVERVIEW OF THE INTERRELATIONSHIPS OF THE THREE AIMS:

As discussed above the program has three interrelated aims. As indicated in the cartoon the first stage of the work involves the recombinant construction of polymerase cDNA into a family of selected expression vectors which in most cases will possess an inducible promoter. As indicated in I (above) the cDNA will be inserted in both sense and antisense orientations and also site-directed mutants. This has been accomplished during the first year. In AIM II we will be concerned with construction (I-D) and expression of site-directed mutants and potential inhibitory peptides *in vivo* in order to eventually modulate the activity of poly(ADP-Rib) polymerase in cells, upon induction during DNA repair. As indicated in II, the various expression vectors will be stably transfected into a variety of eukaryotic cells generally by co-transfection with a selectable gene. As indicated (II-A-C) we might expect various levels of overexpression and underexpression of poly(ADP-Rib) polymerase. In the case of the site-directed mutants and the inhibitory peptides (II D) we anticipate cells with reduced capacity for ADP-ribosylation. Biochemical and molecular biology characterizations (III, above) of the gene products of the various transfected cells are proposed prior to cytotoxicity or DNA repair analysis. These will include: (III-A) Southern analysis to confirm integrated copies of the cDNA; (III B) both Northern and primer extension analysis of cellular mRNA to confirm that upon induction actual expression of the foreign gene occurs; (III-C) immunoprecipitation

of poly(ADP-Rib) polymerase *in vivo* after induction. Finally, using the well characterized cells obtained above a variety of cytotoxicity, mutagenicity DNA repair protocols (IV) will be initiated to indicate the effects on recovery of cells from various DNA damaging as occasioned by environmental toxic agents when a requirement for ADP-ribosylation is encountered.

I VECTOR CONSTRUCTS



II EXPRESSION IN CELLS

A) Endogenous levels

B) 10-20 fold overexpression ("sense") and 3' truncated stable mRNA

C) Reduced levels ("antisense")

D) Reduced activity due to expression of Domain Peptide or site-directed mutant

III BIOCHEMICAL CHARACTERIZATION OF TRANSFECTED CELLS

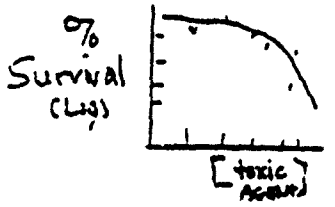
A) Southern analysis for for presence of integrated cDNA copies

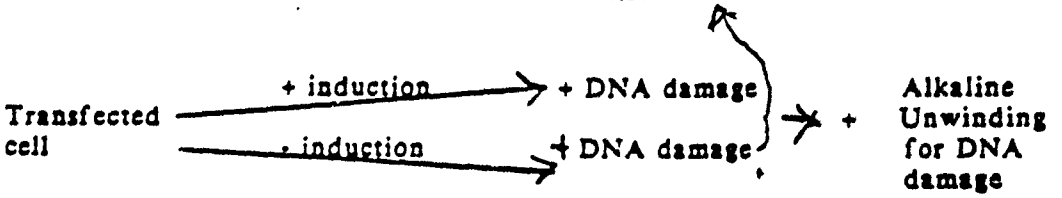
B) Northern and primer extension ± induction

C) ^{35}S Met-IMMPT of protein

D)"Activity" of Polymerase ± inducer

IV CYTOXICITY AND DNA REPAIR





(b). STATUS OF THE RESEARCH EFFORT

PADPRP GENE EXPRESSION STUDIES-RELATIONSHIPS TO FUNCTION

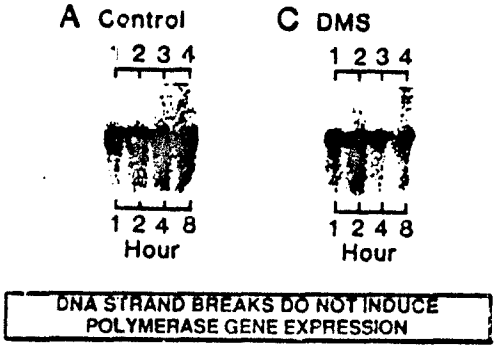
A. PADPRP TRANSCRIPTION IS NOT EFFECTED BY DNA BREAKS

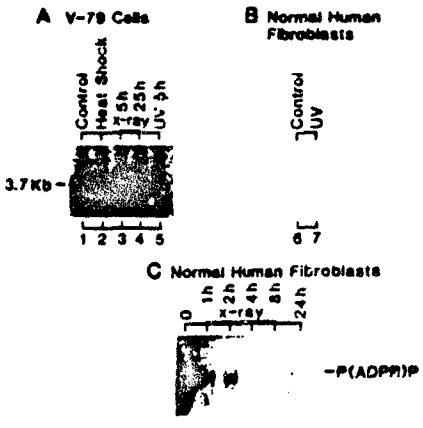
Kishor Bhatia, Albert J. Fornace, Masue Imaizumi, Theodore R. Breitman, Barry W. Cherney, and Mark E. Smulson: The Expression and Overexpression of the Poly ADP-ribose polymerase Gene during DNA Repair and Differentiation: Carcinogenesis (1990) in press.

The catalytic activity of PADPRP is totally dependent upon the presence of DNA strand breaks. Having isolated a full-length cDNA for the polymerase, we subsequently evaluated the effect of endogenously and exogenously induced DNA strand breaks on the transcriptional control of this enzyme.

During retinoic acid or dimethyl sulfoxide-induced differentiation of HL-60 human leukemia cells, which may involve DNA breaks as well as other changes in chromatin, mRNA levels for the polymerase increased very early and remained high for up to 48 after which it decreased to pre-induced levels.

Polymerase transcript levels did not change, however, during the induction of DNA strand breaks by dimethyl sulfate, a variety of other alkylating agents, X irradiation, or UV irradiation in several mammalian cell lines. It appears that in sharp contrast to the catalytic requirement of the polymerase, the induction of transcription of the polymerase gene may not be a strand break-dependent process.





HYPER-EXPRESSION OF PADPRP DURING TRANSIENT TRANSFECTION: EFFECT ON DNA REPAIR RATES

Since PADPRP expression did not respond to DNA damage, we tested the effect of plasmid-induced increases in the transcripts.

The feasibility of genetically modulated PADPRP activity (ie. AIM I below) is illustrated by recent preliminary expression studies performed during the past period . The data below illustrates a DNA transient transfection of Cos cells utilizing pcD-12 and pcD-19, a partial cDNA for PADPRP, which is deleted by approximately 400 bases at the 5' region of the cDNA. After 48 hr the cells were assayed for PADPRP. The data indicates nearly a three-fold increase of specific activity for PADPRP over

endogenous levels was present by the transient transfection. The same extracts analyzed by "activity gel" analysis or by immunoprecipitation with anti-PADPRP (B&C) indicates that here is a 10-15 fold increase in PADPRP enzyme mass in these cells.

We then examined DNA repair in these transfected cells. Cells were irradiated with 200rad (cGy) on ice and immediately allowed to repair at 37°C. In the data illustrated, we employed the alkaline elution method in collaboration with the laboratory of Kurt Kohn (NIH). The differences in the amount of SSB-released during a 15 min repair period in cells hyperexpressing polymerase were compared to control treatments. The data suggests that the DNA repair initial rate in the transfected cells with the sense mRNA was nearly twice that of control cells (i.e. 766 break freq. versus 387). To confirm that the increased rate of induced DNA strand breaks was due to increased poly (ADP-ribosylation) of Cos cells, the effects of the polymerase inhibitor 3-AB was also studied. The inhibitor reduced the increase in repair due to expressed polymerase.

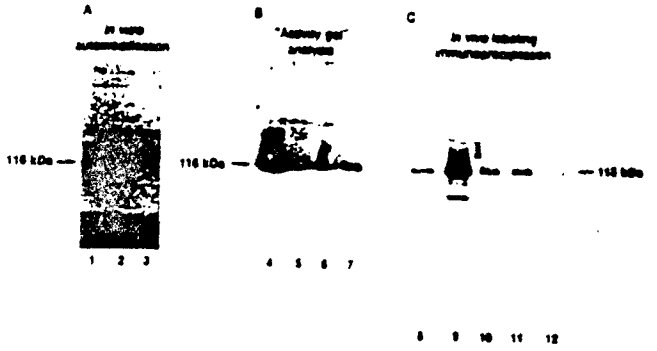


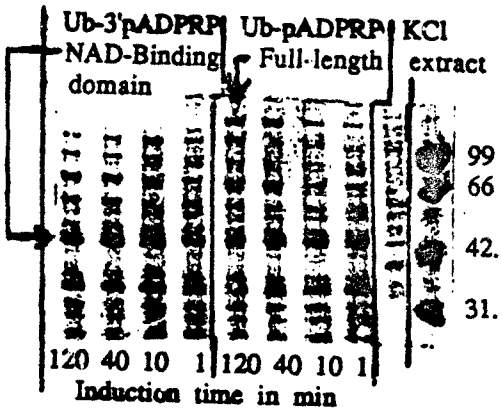
Table 2. Hyper-expression of poly(ADP-ribose) polymerase increases the rate of X-ray induced DNA break resealing in Cos cells.

Cell Treatment	Time (min) to reseal at 37°	DNA Break Frequency (Rad-Equivalents)
None	0	2000
	15	766
Mock-Transfected	0	2000
	15	723
pcD-12	0	2000
	15	387

EXPRESSION OF HUMAN PADPRP IN *E. COLI* AND IN YEAST-RELATIONSHIP TO FUNCTION

Cherney, B., Chaudhry, B., Butt, T., Bhatia, K. and Smulson, M.E.
Expression and analysis of catalyating active and mutant human PADPRP in *E. Coli* : J. Biol. Chem. (1989) Submitted.

Using the bacterial expression vector described in more detail below in AIM I, we have placed the native human PADPRP gene, fused to ubiquitin, in front of the λpL-promoter. By heat induction, we have demonstrated the expression of a catalytically active 113 Kda PADPRP enzyme fused to ubiquitin, as well as several polymerase deletion mutants which are catalytically inactive and will be used for the experiments described in AIM I . A typical induction is shown below:



IN VITRO ACTIVITY OF POLY ADPRP

Ub-Polymerase + 32P-NAD $\xrightarrow[DNA]{Mg^{++}}$ Ub-Polymerase-[ADPR]

UB-Polymerase transfected	cpm X10-4/mg Protein
Non-Induced cells	0.1
Induced Cells	80.0
Induced cells + Polymerase inhibitor 3-Aminobenzamide	57

As shown above by Coomassie stain, detectable amounts of either full-length Ub-polymerase or a polymerase deletion mutant, containing the NAD binding domain, are made within 1 minute of induction. By 40 mins. after induction, approximately 5-20% of the total protein is represented by PADPRP. Enzymatically the PADPRP is nearly indistinguishable from the native enzyme. This conclusion is based on the ADP-ribose acceptor pattern, the apparent Km for NAD and its activation by DNA strand breaks.

It should be noted that in the absence of the ubiquitin portion we were unable to synthesize significant amounts of PADPRP. However, the fusion protein with ubiquitin is catalytically active, made in copious amount and will therefore be quite useful for site-directed mutation studies which are outlined in greater detail in AIM I of this renewal application. A protease is available to specifically cleave the Ub-PADPRP junction.

HUMAN POLY(ADP-RIBOSE) POLYMERASE IS FUNCTIONAL IN SCHIZOSACCHAROMYCES POMBE (MS IN PREP.)

The full length cDNA for human PADPRP has been introduced into the yeast Schizosaccharomyces pombe under the transcriptional control of the SV40 early promoter. A number of haploid stably transformed strains which express the human RNAs constitutively have been generated. Phenotypically, these strains showed an absolute requirement, for normal growth and survival, on the addition of NAD+ precursors to supplement those in the culture media.

B. DETECTION OF A PUTATIVE NEW TUMOR SUPPRESSOR GENE ON CHROMOSOME 13Q33-QTER: RELATIONSHIP TO PADPRP SEQUENCES AT THIS LOCI.

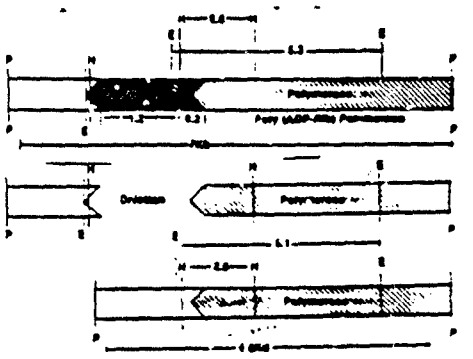
K. Bhatia, I. Magrath, A. Bowcock, K. Huppi, J. Neequayi, J. Cossman, B. Cherney, M.A., DeBernardi, and M. Smulson: A deletion linked to a Poly(ADP-ribose) Polymerase Gene on Chromosome 13q33-qter occurs frequently in the normal Black Population as well as in Tumor DNA (1989) submitted.

Based upon the above human gene mapping data, we embarked on a series of experiments, involving detailed mouse genetics. The laboratory of Michael Potter, at the nearby NIH campus, has extensively characterized various genetic strains of mice which are more (i.e. BALB/c) or less susceptible (i.e. DBA) to plasmacytoma formation, as induced by a microenvironment within the peritoneal cavity *conducive to DNA strand break generation.*

The plasmacytoma resistance gene family cosegregates with the distal end of mouse chromosome 4 and the gene for human PADPRP, as noted above, is located on human chromosome 1, which share large regions of homology with mouse chromosome 4. It was therefore possible that the mouse PADPRP might be a candidate for the resistant gene. During the process of performing mapping for the polymerase for the PADPRP gene in the above mouse strains, in collaboration with the NIH group, a polymorphic *Hind* III band, *only present in strains susceptible to plasmacytoma formation*, was noted. This was subsequently exploited for a linkage analysis with various congenic strains in mice to help ultimately map the chromosome locations of the polymerase in mouse.

Since the atypical PADPRP *Hind III* genotype persisted in animals highly predisposed to B cell tumors, *it seemed logical to seriously investigate whether such structural differences in polymerase genes would be present in the human analog of the MOPC system, namely Burkitt lymphoma.* The data below were obtained by analyzing for PADPRP RFLP in samples of Burkitts lymphoma DNA in collaboration with Dr. Ian Magrath at the NCI.

A



DNA	GENOTYPE AA	AB	BB	TOTAL	Allele Frequency	χ^2	χ^2
NORMAL							
TOTAL	91	24	02	117	0.12		
TUMOR							
B Cell Lymphoma	12	07	04	23	0.33	<0.0001	
Co-Lung	11	09	04	24	0.35	<0.0001	
Co-Breast	34	16	03	53	0.24	<0.05C	
Myeloid Leukemia	11	02	00	13	0.07	<0.45	
Co-Colorectal	44	20	04	68	0.23	<0.0005	
MATCHED NORMAL (From Normal Tissue of Individuals with cancer)							
Co-Lung	84	04	01	89	0.33	<0.012	<0.30
Co-Breast	13	10	00	31	0.30	<0.0005	<0.305
Co-Colorectal	49	19	04	83	0.21	<0.005	<0.70

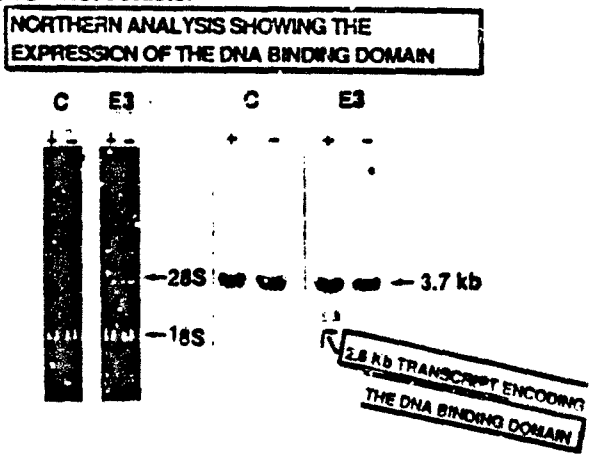
Recent Progress of a Partial Functional Domain for PADPRP and its Effect on Catalytic Activity in Cells

These experiments were performed during the last year and constitute a pilot research example of the types of experiments that will be done to measure modulated PADPR activity and toxic agents during this granting period.

Construction of pBS-1 Plasmid. A 1.88 kb fragment encompassing the whole DNA binding domain and part of automodification domain of human PADPRP cDNA was purified from pCD12, the plasmid containing the original full length clone for poly ADP-Rib polymerase. Such a fragment, extending from the Bam H1 site within the SV40 early region promoter to the Bam H1 site at the nucleotide position 1599 into the PADPRP coding region was subcloned in both sense and antisense orientations into the unique BglII site of the expression vector p341-3. This vector allows the inducible expression under the the control of mouse metallothionein I promoter, and provides adequate signals for transcription and translation termination within the SV40 early polyadenylation region located immediately downstream from the BglII cloning site. Subclones were isolated by transformation into *E.coli* C600. Plasmid DNA was purified by double CsCl density gradient centrifugation.

Isolation of Mouse Cell Lines Expressing the DNA Binding Domain of the Human Poly(ADP-Rib)polymerase. NIH/3T3 fibroblast cells ($5x10^5$) were transfected with a DNA mixture of the recombinant expression vector having the inserted Bam HI fragment in sense orientation and pSV2neo which carries the G_{418} selectable marker in molar ratio of 40:1. Control cells were transfected with p343-3 and the pSV2neo in the same molar ratio. Transfection was done by modified calcium phosphate precipitation method. Transfected cells were selected for G418 resistance 48 hours post-transfection. After two weeks of G_{418} selection, 20 individual clones were picked up and expanded into individual cell lines. The remaining colonies were trypsinized and expanded as pool cell line.

Northern Blot Analysis of the Human mRNA in NIH/3T3 Cells. In Northern analysis, total RNA from three representative clones, (E3, E8 and E9) after electrophoresis was blotted onto Zeta probe membrane. The cells were grown in DMEM containing 10% dialyzed FCS supplemented with ITS. Induction of cells growing in log phase was performed for 16-18 h with zinc and cadmium at 50 and 0.5 μM final concentrations respectively. A human PADPRP cDNA insert hybridized with approximately 2.6-kb mRNA (Figure 3) both in the presence (+) (lanes 2, 4, and 6) and absence (-) (lanes 1, 3, and 5) of induction. It appears from these results that the mouse metallothionein I promoter of the expression vector is leaky under this growth condition of NIH/ 3T3 fibroblasts.



Poly(ADP-Rib)polymerase Activity. E3 and C cells were grown in conditioned medium and induced for 16-18 h and were made permeable to nucleotide by standard method. ADP-ribose incorporation was measured in acid-insoluble material by filter binding assay. We noted a significantly higher ADP-ribosylation in E3 cells compared with C cells (Table 1).

Specific Activities of Poly(ADP-ribose) polymerase

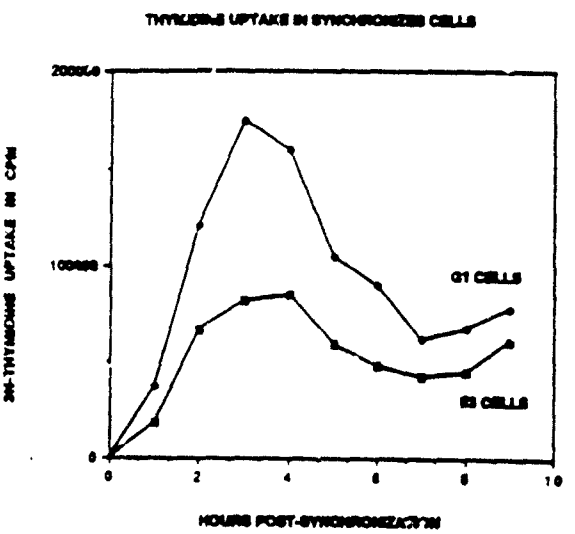
Cells	Induction	pmol ADP-Rib/min/mg protein		
		Permeabilized Cells		Sonicated cell extracts
		-DNase	+DNase	
Control	-	161±22	3908±639	931±72
	+	173±15	3744±617	923±58
Experimental	-	258±21	2781±698	980±84
	+	319±29	3375±540	884±38

Similar activation was also noted with other clones compared with C cells (data not shown). However, when the permeabilized cells were preincubated with DNase I (200 µg/mL) in ice for 10 min the difference in the activities between E3 and C cells was almost abolished. In contrast, in an *in vitro* assay of ADP-ribosylation using sonicated cell extract we found no significant difference in activity between E3 and C cells (Table 1).

These experiments were unexpected in the sense that we anticipated that expression of this analogue truncated part of the enzyme would inhibit activity. However, the stimulation of activity is quite significant and will be usefully exploited in various strategies later in this project.

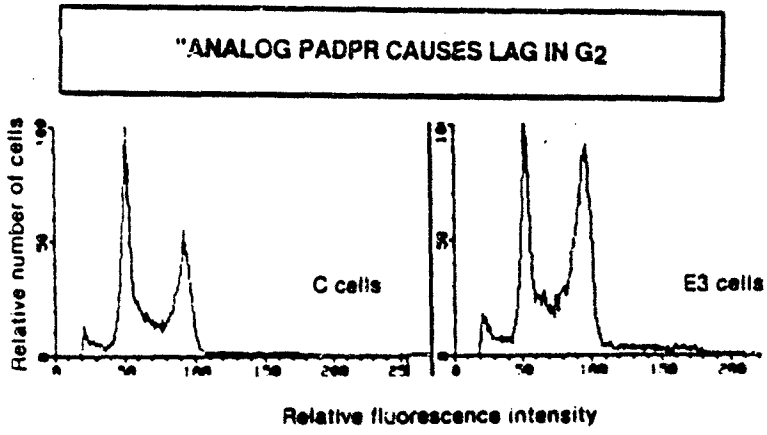
Growth Kinetics. During the first year, we have noted that the clones expressing the tail-truncated human PADPRP have quite reduced proliferative capacity compared with control or parental NIH/3T3 cells. Accordingly, we examined the growth rate of one representative clone (E3) as well as C cells. When density-arrested cells were diluted into fresh medium to allow reentry into the cell cycle E3 cells exhibited slower initial growth rate compared with the control cells.

DNA Synthesis in Synchronized Cells. E3 and C cells were synchronized by double thymidine block according to the standard method and the cells were released to enter the S phase. The rate of DNA synthesis was monitored by incubating the cells in triplicate on a 35-mm plate with 2 µCi of ^{3}H-thymidine in 2 mL of medium for 1 h at 37°C and by measuring the incorporation of radioactivity in trichloroacetic acetic acid insoluble materials at 1-h intervals up to 9 h. It appears from the data that there is no significant difference in the length of the S phase between E3 and C cells. However, the total radioactive thymidine incorporation in E3 cells are remarkably lower than that in C cells. The cells were counted after release of thymidine block, and the data were normalized in terms of number of cells We noted that the initial rate of ^{3}H-thymidine incorporation is very similar if not same. Therefore, the difference in the total amount of thymidine uptake is due to the difference in the number of cells in the plate, although the experiment was started with the same number of cells in each plate. These data clearly indicates that E3 cells exhibited differential growth patterns in different stages of proliferation compared with C cells.



Fluorescence Activated Cell Sorter Analysis. During the first year we initiated the use of fluorescence activated cell sorter analyses to confirm the proliferative stage

specific differential growth patterns of the cells expressing the tail-truncated PADPRP. DNA per cell distribution, histograms of cells, harvested from early and mid-log phases, stained with propidium iodide after fixation in ethanol and RNase digestion are shown below. Both sets of samples were analyzed under identical settings of laser power,electronic amplification and optics. The histograms of DNA from cells, harvested in early log phase reveal that the distributions of E3 cells in G_2 phase is significantly higher than that of C cells, while the E3 cells harvested from mid exponential phase exhibited no difference in cell cycle distributions compared to the corresponding control cells.



We have developed a model system for molecular perturbation of this protein modification system by expressing a genetically engineered N-terminal partial human PADPRP cDNA in murine fibroblast cells during the first year. Such a tail-truncated cDNA under the control of mouse metallothionein I promoter encodes the whole DNA binding and a part of automodification domains. We rationalized that if this partial polypeptide, catalytically inactive, but capable of binding to DNA strand breaks, will interfere with the function of the endogenous enzyme *in vivo*, we might be able to perturb at the molecular levels the various functions of PADPRP

Previously it has been shown by this laboratory that PADPRP mRNA accumulates during the late S phase in synchronized HeLa cells, suggesting a possible requirement of de novo PADPRP in late S and/or early G_2 phases. However, it is unclear whether this role of PADPRP is catalytic or structural. Our observation indicates that NIH/3T3 cells expressing the DNA binding domain of human PADPRP grow at slower rate in early exponential phase compared with C cells or parental NIH/3T3. Detailed cell cycle analyses of these cells during this first year demonstrate that this is due to an arrest in G_2 phase. This G_2 arrest results from the possible interference with the normal function of endogenous PADPRP by the tail-truncated polypeptide. However, the transfectants are able to overcome this growth arrest in the latter phases of growth. One reason may be that the cells are able to reverse the G_2 arrest by an activation of the endogenous enzyme, as we observed an increase in polymer synthesis in permeabilized cells, harvested in in mid log phase. Or alternatively, the cells develop a compensating mechanism to restore the normal growth status. The expression of tail-truncated polypeptide may have important implications for future studies of the project on DNA repair of toxic agents.

C. CUMULATIVE CHRONOLOGICAL LIST OF WRITTEN PUBLICATIONS AND TECHNICAL JOURNALS

PUBLICATIONS

Thraves, P.J., Kasid, U., and **Smulson, M.E.**; Selective Isolation of Domains of Chromatin Proximal to both Carcinogen-Induced DNA Damage and Poly(Adenosine-Diphosphate-Ribosylation), Cancer Research, 45, 386-391 (1985).

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Cherney, B. W., Chaudhry, B., Butt, T., Bhatia, K. and Smulson, M.E. Expression and analysis of catalytically active and mutant human PADPRP in *E. Coli*. J. Biol. Chem. (1989) submitted.

Bhatia, K. Kang, V., Stein, G.S., Cherney, B.W., Haque, S.J., Bustin, M., Huppi, K., and Smulson, M.E. Promoter-Independent and Faithful Cell Cycle Regulation of an Exogenous Human Poly (ADP-Ribose) Polymerase cDNA Expressed in Murine Cells: J. Biol. Chem. (submitted).

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D. LIST OF PROFESSIONAL PERSONNEL

Mark E. Smulson, Ph.D. Scott Simpson, MS (20% Barry Cherney, Ph.D. (20%)

J. Haque, Ph.D. (100%) Kishor Bhatia, Ph.D. (20%) Noelle Lewis (20%)

E. INTERACTIONS (COUPLING ACTIVITIES)

RECENT INVITED SYMPOSIUM CHAPTERS AND LECTURES

Smulson, M.E.: "The Poly (ADP-ribose) polymerase gene: Direct or indirect Involvement in DNA Repair and Malignancy? Fifth International Conference on Environmental Mutagens, Cleveland, OH, July 10-14 (1989).

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