

AD _____

GRANT NUMBER DAMD17-96-1-6109

TITLE: The Possible Role of E2F in Rat Mammary Carcinogenesis

PRINCIPAL INVESTIGATOR: Peggy J. Farnham, Ph.D.
Traci Lee

CONTRACTING ORGANIZATION: University of Wisconsin System
Madison, WI 53706

REPORT DATE: August 1999

TYPE OF REPORT: Annual Summary

PREPARED FOR:
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

DTIC QUALITY INSPECTED 4

20000111 037

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE August 1999	3. REPORT TYPE AND DATES COVERED Annual Summary (15 Jul 96 - 14 Jul 99)	
4. TITLE AND SUBTITLE The Possible Role of E2F in Rat Mammary Carcinogenesis			5. FUNDING NUMBERS DAMD17-96-1-6109	
6. AUTHOR(S) Peggy J. Farnham, Ph.D. Traci Lee				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Wisconsin System Madison, WI 53706			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Frederick, Maryland 21702-5012			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200) Upregulation of the E2F family of transcription factors has been suggested to be commonly associated with a neoplastic phenotype. To understand the consequences of altered E2F activity in cancer, I have used two model systems: a rat mammary tumor assay and an NIH 3T3 focus formation assay. While the rat mammary study did not yield conclusive results, the tissue culture assay revealed that E2F transcription factors are not oncogenic in NIH 3T3 cells. Rather, overexpression of E2F1, E2F2, and E2F3 caused a dramatic decrease in foci induced by the activated c-Ha-ras oncogene. This inhibition of ras-mediated transformation was dependent upon E2F DNA binding activity but did not require amino or carboxy terminal E2F1 protein interaction domains. I have found that introduction of exogenous E2F1 into NIH 3T3 cells previously transformed by ras was less growth inhibitory than was E2F1 in untransformed NIH 3T3 cells, suggesting that neoplastic transformation can partially overcome the inhibitory effects of E2F1. However, ras transformed cells remain sensitive to the inhibitory effects of E2F2 and E2F3. I am currently investigating if E2F can inhibit transformation mediated by oncogenes other than the ras oncogene.				
14. SUBJECT TERMS Breast Cancer E2F transcription factors, ras, oncogene, transformation, growth inhibition, NIH 3T3, focus formation			15. NUMBER OF PAGES 13	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

✓
44. ____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Reyner Farlan 8/6/99
PI - Signature Date

GRADUATE STUDENT SIGNATURE

3 ↳ Traci Lee 8/6/99

TABLE OF CONTENTS

	PAGE NUMBER
I. FRONT COVER	1
II. STANDARD FORM	2
III. FOREWORD	3
IV. TABLE OF CONTENTS	4
V. INTRODUCTION	5
VI. BODY	6
VIII. REFERENCES	12
IX. APPENDIX	13

INTRODUCTION

Many factors which control the G1 to S phase transition of the cell cycle are implicated in the formation of cancer. The activation of several G1/S-phase regulated genes is mediated via a family of transcription factors called E2F. The abundance and activity of the E2F transcription factors are tightly regulated by positive and negative regulators of the G1 to S phase transition. These regulators are often mutated in breast cancer in such a way that they could upregulate E2F activity. The frequent deregulation of the Rb/cyclin signal transduction pathway in human cancers has led to the hypothesis that increased E2F activity is a critical determinant of the neoplastic phenotype. If so, intentional overexpression of E2F family members should be able to cause neoplastic transformation. Several studies have demonstrated the ability of E2F family members to act as oncogenes. Although these overexpression studies clearly indicate that E2F1 has the potential to be an oncogene under certain conditions, the creation of E2F1 null mice has provided an alternative view. Mice lacking E2F1 develop tumors in certain organs after about a year, suggesting that loss of E2F1 enhances tumor development, a hallmark of a tumor suppressor gene, not an oncogene.

Because E2F1 has been shown to both enhance and inhibit tumor formation, it is apparent that the effects of this protein on cell growth control are exquisitely sensitive to others factors such as cell type and the presence or absence of cooperating or inhibitory proteins. Understanding the mechanisms by which E2F1 can influence cell proliferation will require a detailed analysis of such parameters. Therefore, I have undertaken a study of the role of E2F1 in two model systems: 1) an animal model system for mammary carcinogenesis, in which retroviruses which overexpress a gene can infect the epithelial cells lining the mammary duct of a rat and cause tumor formation depending on the oncogenic activity of the overexpressed gene, and 2) a well characterized cell culture model system in which neoplasia is mimicked by focus formation. While studies from the animal study was inconclusive (see 1997 Army annual report), I have found that E2F family members have a tumor suppressor phenotype in this system, I delineated the domains of E2F1 required for this inhibition, and further characterized the nature of the growth inhibition.

BODY

SUMMARY OF RESULTS

E2F4 retroviruses do not cause tumors in the rat mammary gland.

As summarized in my 1996-97 annual report, I was unable to create retroviruses that visibly overexpressed the mouse E2F1 protein. Therefore, I concentrated my efforts to make E2F4 retroviruses which caused overexpression of E2F4 in NIH 3T3 cells upon infection. These retroviruses were placed into the rat mammary gland. While my positive control for tumor formation, retroviruses which express the ras oncogene, caused several tumors, arising as early as one month post-infection, I was unable to detect palpable tumors or abnormal histological growth within the rat mammary glands which were infected with the E2F4 retroviruses as late as eight months post-infection.

Rat mammary studies investigating the effect of E2F overexpression on ras mediated tumor formation could not be continued due to technical problems.

As summarized in my 1997-98 annual report, I created different retroviral constructs to coexpress E2F transcription factors with the ras oncogene in the hopes of determining if E2F transcription factors could increase or decrease the number of tumors caused by the ras oncogene. Unfortunately, retroviruses which should coexpress E2F and ras did not express a functional activated ras protein. Due to this problem, I decided to address the same issues in my Statement of Work using the NIH 3T3 focus formation assay as a tissue culture model system for cancer instead of using the rat mammary system.

1998-99 KEY RESEARCH ACCOMPLISHMENTS

- E2F transcription factors do not cause NIH 3T3 focus formation.
- E2F transcription factors inhibit NIH 3T3 focus formation mediated by activated c-Ha-ras .
- DNA binding ability appears to be both necessary and sufficient for E2F1 inhibition of ras transformation.
- Ras transformed cells are partially resistant to E2F1 inhibitory effects.
- E2F1 protein is functionally expressed in ras plus E2F1 expanded foci.
- Increased E2F1 in NIH 3T3 cells is growth inhibitory.

1998-99 REPORTABLE OUTCOMES

- Conference poster presentation: "DNA binding competent E2F1 can inhibit ras mediated transformation without inhibiting cell cycle progression" by Lee, T.A. and Farnham, P.J. at the AACR "Gene Regulation and Cancer" Special Conference in Cancer Research in Hot Springs, VA. Oct. 14-18th, 1998
- Seminar presentation: "The involvement of E2F in ras-mediated transformation and in the regulation of DNA replication" UW-Madison, Cancer Biology Seminar Series. Oct. 22nd, 1998.
- Manuscript of 1998-99 results is in preparation for publication submission within the next three months.
- Ph.D. Thesis Defense will be in November, 1999.

1998-99 RESULTS

E2F transcription factors do not cause NIH 3T3 focus formation but instead inhibit NIH 3T3 focus formation mediated by activated c-Ha-ras .

To test the effects of E2F transcription factors on focus formation, plasmids expressing E2F1, E2F2, E2F3, E2F4, E2F5, or empty vector controls were transfected into NIH 3T3 cells with or without a plasmid expressing the activated c-Ha-ras oncogene. Cells were allowed to grow for 14 days, after which time, cells were fixed, stained, and scored for transformed foci. No foci were observed in plates transfected with E2F plasmids alone, suggesting that E2F family members do not have oncogenic potential in this assay. However, cotransfection of E2F with ras caused a decrease in ras mediated focus formation. E2F1, E2F2, or E2F3 could cause a 70-80% decrease in the number of ras mediated foci, with less inhibition detected using E2F4 or E2F5. To determine whether the lower inhibitory effect seen by E2F4 and E2F5 was due to differences in protein expression, the focus assay was repeated using E2F1, E2F2, E2F3, E2F4, and E2F5 expression constructs which were all fused to a hemagglutinin (HA) tag. The HA tagged E2F proteins were found to have similar ras inhibitory effects as the E2F proteins produced by the different vectors. Western blot analysis indicate that the inability of E2F4 and E2F5 to inhibit focus formation was not due to low protein expression; the amounts of E2F4 and E2F5 were as high or higher than amounts of E2F1, 2, and 3.

DNA binding ability appears to be both necessary and sufficient for E2F1 inhibition of ras transformation.

E2F proteins have several different properties, including DNA binding, transactivation, and protein-protein interactions. To determine which properties of E2F were important for inhibition of transformation, I concentrated on the best characterized E2F family member, E2F1. Previous studies have identified specific amino acids critical for DNA binding, transactivation, and binding to the Rb family of transcriptional repressors. To test whether inhibition of ras required E2F to bind to DNA, a mutant E2F1 protein called E2F1 E138 which contains an inactivating mutation in

the DNA binding domain was tested in the focus formation assay. E2F1 E138 could not inhibit ras mediated focus formation, indicating that DNA binding properties of E2F1 are critical for ras inhibition. To determine if E2F inhibition of ras transformation was dependent on the ability of E2F to transactivate or to bind to Rb, a vector expressing a mutant E2F1 protein missing the last 20 amino acids, E2F1 Δ 417, was utilized. E2F1 Δ 417, which lacks a pocket binding domain and most of the transactivation domain, effectively interfered with ras in the focus assay indicating that Rb binding and transactivation by E2F1 are not necessary. To ensure that the loss of inhibition was not due to the lack of expression of the mutant protein, an E2F1 Western was performed on NIH 3T3 cells lipofected with the wildtype and mutant E2F1 expression constructs. Therefore, DNA binding is a necessary function of E2F1 in inhibiting ras-mediated transformation of NIH 3T3 cells.

Although the carboxy terminal protein interaction domain was not required for E2F1 to inhibit ras, there are several other regions of E2F1 that mediate protein-protein interactions. Therefore, my next step was to perform a more detailed analysis of E2F1. E2F1, E2F2, and E2F3 share similar amino terminal sequences not found in E2F4 or E2F5. These domains mediate interactions with proteins such as cyclin A and the Sp1 transcription factor (12, 14, 15). It was possible that such protein-protein interactions specify the higher inhibitory potential of E2F1, 2 and 3, as compared to E2F4 and E2F5. To address whether amino terminal sequences are important in ras inhibition, we utilized an E2F1 amino-terminal deletion mutant, E2F1 88-437, which contains a truncation of the first 88 amino acids, and E2F1 Δ 79-103, a mutant E2F1 containing an internal deletion within the cyclin A binding domain. My results indicate that cyclin A binding is not required for inhibition of ras transformation. In fact, loss of cyclin A binding causes E2F1 to be a more effective inhibitor of transformation. Since cyclin A is thought to regulate DNA binding by E2F1 through the cell cycle (2), loss of cyclin A binding may allow E2F1 to bind to the DNA better, especially at inappropriate times in the cell cycle. While deletion of the first 88 amino acids of E2F1 does decrease the effectiveness of E2F1 as an inhibitor of transformation, E2F1 88-437 still retains the ability to reduce ras-mediated foci to 37.5% of normal levels. The first 88 amino acids contain the Sp1 transcription factor interaction domain as well as amino acids which contribute to nuclear localization (1, 3, 4). Either of these functions may contribute to the inhibition of focus formation. I next further analyzed the region downstream of the DNA binding domain. While consecutive deletions up through the dimerization domain of E2F1 caused decreases in inhibition, deletion of carboxy sequences up to amino acid 191 still resulted in inhibition of ras transformation to 30% of the number of foci seen with ras alone. Thus, the minimal region common to all inhibitory E2F1 proteins is amino acids 103-191. Included within this region is amino acids 90-191 which has been shown to be the minimal binding domain using *in vitro* gel shift assays (6).

As noted above, the different E2F family members affect ras transformation to different extents. For example, E2F1 has a much greater ability to inhibit ras-mediated foci formation than does E2F4. However, my deletion analysis indicates that the DNA binding domain of E2F1, which is very similar to the DNA binding domain of E2F4, appears to be the only critical component for inhibition of ras transformation. Therefore, one might expect that E2F4 would have the ability to inhibit ras as well as E2F1. It has been previously noted that overexpressed E2F1 is found in the nucleus whereas overexpressed E2F4 is found in the cytoplasm (7). To determine if a difference in cellular localization affected the ability of E2F4 to inhibit the ras oncogene, I utilized an E2F4 protein having a consensus nuclear localization signal from the SV40 large T antigen attached to the amino terminus (7). The addition of a nuclear localization signal to E2F4 enabled this family member to inhibit ras transformation as effectively as E2F1. Thus it seems as if the critical determinant in inhibition of ras mediated focus formation is to get sufficient quantities of a DNA binding competent E2F to the nucleus.

Ras transformed cells are partially resistant to E2F1 inhibitory effects.

I next wished to characterize the mechanism by which E2F family members were inhibiting ras transformation. As a first step, I examined whether the growth inhibitory effect was specific for the ras-transfected population. I transfected NIH 3T3 cells with E2F expression vectors plus plasmids which confer hygromycin B resistance, and allowed colonies to grow under hygromycin B selection for 2 weeks. E2F1, E2F2, and E2F3, but not E2F4 or E2F5, caused a dramatic reduction in colony formation. Also, I found a similar profile of colony inhibition for both wildtype and mutant E2F1 proteins as seen with foci inhibition. These results suggest that the effects of E2F on NIH 3T3 cells are not specific to the ras transformation assay. Rather, overexpression of E2F appears to be inhibitory to cell growth in general.

Many regulators of E2F activity are deregulated in human cancers, leading to a commonly held hypothesis that tumors should display an increase in E2F activity. Therefore, it was a bit surprising that overexpression of E2F proteins is inhibitory to cell growth. However, it is possible that deregulation of E2F activity may be a late event in neoplastic transformation and that increased levels of E2F is not inhibitory to the growth of transformed cells. To test this possibility, multiple foci derived from ras transformed NIH 3T3 cells were expanded into cell populations. These expanded foci were tested for the effects of E2F1 in a colony formation assay using wt E2F1, E2F1 Δ 417, and E2F1 E138. I found that, instead of the 80% inhibition witnessed with untransformed NIH 3T3 cells, ras transformed cells are now only inhibited 50% compared to colonies formed with empty vector. Therefore the transformation process allowed cells to partially overcome inhibition by E2F1. This suggests that overexpression of E2F1 may be more detrimental to normal cells than to transformed cells.

E2F2 and E2F3, however, were still potent inhibitors of colony formation, suggesting a different mode of inhibition for E2F2 and E2F3 than for E2F1.

E2F1 protein is functionally expressed in ras plus E2F1 expanded foci.

Although E2F1 is able to cause a five fold decrease in focus formation by activated ras in normal NIH 3T3 cells, a small number of transformed foci are still detected. Three possibilities for the appearance of these foci are as follows: (1) the foci have selected against the expression of E2F1, the inhibiting activity, (2) the foci have selected for increased expression of ras, the transforming activity, or (3) the foci arose from cells which could bypass the E2F1 inhibition, without affecting E2F1 or ras expression. To distinguish among these possibilities, foci were analyzed for the expression of wildtype E2F1, E2F1 Δ 417, or E2F1 E138. Multiple foci arising from the focus assays with ras plus E2F1, or ras plus E2F1 E138 were picked and expanded. As a control, foci from ras alone plates were also analyzed. Nuclear extracts from the expanded foci were then used in a Western blot. Since the E2F1 expression vectors produce human E2F1 protein and the KH95 E2F1 monoclonal antibody used cannot effectively detect mouse E2F1, we were able to distinguish which foci maintained exogenous expression of E2F1. When ras foci from empty vector plates were analyzed by Western analysis, no exogenous E2F1 was detected; however, five of the eleven foci expressed E2F1 in the plates transfected with wildtype E2F1 plus ras. Six of eleven ras/E2F1 E138 expanded foci expressed E2F1 E138. Since a similar number of expanded ras foci continue to express either E2F1 or E2F1 E138, there appears to be no selection against the inhibiting form (wildtype E2F1) versus the noninhibiting form (E2F1 E138) of E2F1 in the ras transformation process.

To determine if the E2F1 expressed in these foci is still functional, whole cell extracts from foci shown to overexpress E2F1 was tested in a electrophoretic mobility shift assay. An additional band of E2F gel shift activity was detected in extracts from expanded E2F1/ras foci but not in CMV/ras foci. The mobility of this band indicates that E2F must be in a complex with other proteins. Even though the extra E2F binding activity was not disrupted or supershifted with E2F1 antibodies, it may be that the epitope for the antibody is masked by other proteins interacting with E2F1. Consistent with this idea, Singh et al reported a similar extra band in extracts of rat embryo fibroblasts transformed by E2F1 which could not be supershifted by E2F1 antibodies (5). Therefore, there is good evidence to support that the E2F1 expressed in the foci from the E2F1/ras focus assays remains functional.

To determine if there is a disproportionate expression of the ras protein in the foci that arose in the presence of a Western on whole cell extracts from a panel of different expanded foci was performed using a anti-pan-ras mononclonal antibody. Results indicate similar levels of ras expression in all the expanded foci regardless of the expression of E2F1.

Therefore, it appears as if the cells that escaped E2F1-mediated growth inhibition did so via a mechanism independent of E2F1 or ras levels.

Increased E2F1 in NIH 3T3 cells is growth inhibitory

I wished to characterize the mechanism by which E2F family members mediate growth inhibitory effects in NIH 3T3 cells. I therefore analyzed the cell cycle of NIH 3T3 cells transfected with wildtype E2F1, E2F1 Δ 417, and E2F1 E138 by flow cytometry. The E2F1 transfected population was selected by transfecting the cells with a GFP expression plasmid in a one to five ratio with E2F1 expression plasmids. DNA profiles for the GFP positive population, which should be E2F1 positive as well, indicated that introduction of wildtype or mutant E2F1 did not cause a change in the percentage of cells in G1, S, or G2/M. Interestingly, however, I did detect a small difference in the percentage of GFP positive cells within the total cell population for the different transfected groups. GFP only labels live cells so decreases in the percentage of GFP positive cells may indicate toxicity. While cotransfection of GFP with empty vector gave rise to a 12.5% GFP positive population, I witnessed an 8% and 9% GFP positive population for wt E2F1 and E2F1 Δ 417, respectively, and 18.5% for E2F1 E138. The decreases in the number of GFP positive cells after transfection with E2F1 may be related to the ability of E2F1 to inhibit focus formation. While these results are not as dramatic as the results seen in the focus assay, the differences may be due to the differences in the lengths of the experiments. In the GFP assay, cells are harvested two days post transfection and in the colony formation and focus formation assays, cells are harvested two weeks following transfection. It is possible that a slight decrease in cell number in a short term assay will become a larger decrease in a long term assay. I am currently conducting other assays to investigate whether these cells are undergoing apoptosis.

REFERENCES

1. **Karlseder, J., H. Rotheneder, and E. Wintersberger.** 1996. Interaction of Sp1 with the growth- and cell cycle-regulated transcription factor E2F. *Mol. Cell. Biol.* **16**:1659-1667.
2. **Krek, W., G. Xu, and D. M. Livingston.** 1995. Cyclin A-kinase regulation of E2F-1 DNA binding function underlies suppression of an S phase checkpoint. *Cell* **83**:1149-1158.
3. **Lin, S.-Y., A. R. Black, D. Kostic, S. Pajovic, C. N. Hoover, and J. C. Azizkhan.** 1996. Cell cycle-regulated association of E2F1 and Sp1 is related to their functional interaction. *Mol. Cell. Biol.* **16**:1668-1675.
4. **Müller, H., M. Moroni, E. Vigo, B. O. Peterson, J. Barteck, and K. Helin.** 1997. Induction of S-phase entry by E2F transcription factors depends on their nuclear localization. *Mol. Cell. Biol.* **17**:5508-5520.
5. **Singh, P., S. H. Wong, and W. Hong.** 1994. Overexpression of E2F-1 in rat embryo fibroblasts leads to neoplastic transformation. *EMBO J.* **13**:3329-3338.
6. **Ivey-Hoyle, M., R. Conroy, H. E. Huber, P. J. Goodhart, A. Oliff, and D. C. Heimbrook.** 1993. Cloning and characterization of E2F-2, a novel protein with the biochemical properties of transcription factor E2F. *Mol. Cell. Biol.* **13**:7802-7812.
7. **Lindeman, G. J., S. Gaubatz, D. M. Livingston, and D. Ginsberg.** 1997. The subcellular localization of E2F-4 is cell cycle dependent. *Proc. Natl. Acad. Sci. U.S.A.* **94**:5095-5100.

APPENDIX

Abstract submitted for poster presentation, "DNA binding competent E2F1 can inhibit ras mediated transformation without inhibiting cell cycle progression" by Lee, T.A. and Farnham, P.J. at the AACR "Gene Regulation and Cancer" Special Conference in Cancer Research in Hot Springs, VA. Oct. 14-18th, 1998:

E2F transcription factors are believed to be responsible for the transcriptional regulation of a number of DNA replication and cell cycle regulation genes. Both loss and overexpression of E2F1 have been shown to cause transformation of certain cell types *in vivo*. In this respect, E2F1 has properties of an oncoprotein and a tumor suppressor protein. In order to better understand the role of E2F1 in transformation, we are characterizing wildtype and mutant forms of E2F1 in a ras mediated transformation assay. We have found that both wt E2F1 and the E2F1 del417-437 mutant (no transactivation or Rb binding) interfered with ras mediated transformation while the E2F1 E138 mutant (no DNA binding) could not. In order to investigate how this occurred, we looked at the effect of del417 on cell cycle kinetics. DNA profiles for NIH 3T3 cells transfected with del417 looked normal compared to vector alone for log growing cells. In addition, we see the same percentage of cells transfected with del417 and with vector alone suggesting that apoptosis is not playing a role in inhibiting ras transformation. We suggest that E2F1 del417 is affecting transformation specific E2F targets. Assuming E2F target gene specificity, overexpression of E2F1 may prevent other E2F family members from binding to these critical targets. We are currently investigating E2F target gene specificity using a chromatin immunoprecipitation assay.