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Award Number: DAMD17-00-1-0039

TITLE: The Roles of the Y Chromosome Genes in Prostate Cancer

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REPORT DATE: February 2003

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	February 2003	Annual (15 Jan 02 - 14 Jan 03)

4. TITLE AND SUBTITLE
The Roles of the Y Chromosome Genes in Prostate Cancer

5. FUNDING NUMBERS
DAMD17-00-1-0039

6. AUTHOR(S) :
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, 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 Words)*** *(abstract should contain no proprietary or confidential information)*

Currently we lack information on the role of the men-only chromosome, the Y chromosome, on prostate cancer. This project is designed to address this problem and to identify candidate genes on the Y chromosome involved in this male-specific cancer. The objectives are: 1) to study the expression of Y chromosome genes in prostate cancer and 2) to evaluate their effects in over-expression in the prostate of transgenic mice. We have completed a survey on the expression of 31 Y chromosome genes in prostate cancer and have identified TSPY gene to be the most likely one to play a role in male oncogenesis. We have isolated and sequenced several polymorphic TSPY transcripts in normal and cancerous prostate samples, demonstrated that over-expression of TSPY potentiates cell proliferation in vitro and tumor formation in vivo. TSPY interacts with the mitotic cyclin B. Its expression is influenced by androgen. For the no-cost extension period, we hope to finish our transgenic study in over-expressing this and other Y chromosome gene(s) in prostates of mice and to evaluate its effects in prostatic oncogenesis and to define the TSPY domain(s) important for its interaction with the mitotic cylin B. These studies will provide important information on the role of this male-specific chromosome on prostate cancer development and treatments.

14. SUBJECT TERMS:	15. NUMBER OF PAGES
Y chromosome, prostate cancer, transgenic mouse, molecular genetics	12
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

The male-specific chromosome, Y chromosome, plays critical roles in the physiology of male organs, such as testis and prostates. Abnormalities or mutations among genes on this chromosome are expected to have significant influences on disease development, such as infertility, and men-only cancers of the testis and prostate (1). There are two specific objectives for the present project. The first one is to identify the most likely candidate proto-oncogene(s) among the genes on this chromosome using a series of expression studies. The second one is to evaluate the role(s) of the identified candidate(s) in oncogenic activities in transgenic mice, particularly on the prostate cancer. These studies should provide valuable information on the potential role of the human Y chromosome in prostatic oncogenesis.

BODY

Task 1. To identify the candidate gene on the Y chromosome that plays a role in prostate cancer

Studies under this task have already been accomplished during Year 1 and 2. The results have been communicated in reports of the previous years of this project. Briefly, we had studies a total of 31 genes on the Y chromosome in a panel of prostatic samples consisting of normal and cancerous tissues. The results identified one strong candidate gene, TSPY that is expressed at high levels in prostate cancer. Its expression increases with increasing degrees of malignancy (2). TSPY is also a candidate gene for the gonadoblastoma locus on the Y chromosome (GBY) that predisposes the dysgenetic gonads of XY sex-reversed females to cancer development (1,3,4).

Task 2. Functional evaluation of Y chromosome genes in prostate cancer

The identification of a strong candidate gene, TSPY, from the Y chromosome that may play a role in prostatic oncogenesis has led us to focus on defining the probable function of its over-expression in prostate cancers. Previous studies under this task further demonstrated that over-expression of the TSPY gene potentiates cell proliferation in culture and tumorigenesis in nude mice (5). Studies on its expression profile in prostate cancer demonstrated that there are polymorphic forms of this gene, resulting in a spectrum of TSPY variant proteins. All TSPY variant proteins harbor a conserved domain homologous to those present in a family of cyclin B binding proteins, such as NAP-1 and SET oncoportein, involved in cell cycle regulation (6-9). These results are significant in terms of a potential role of cell cycle regulation of TSPY, abnormalities of which might be involved in the disruption of such regulatory mechanism in male tumor cells.

Our studies in Year 3 were focused on defining the possible interaction between TSPY and the mitotic cyclin B, its regulation by androgen, and on evaluating its role in prostatic oncogenesis in transgenic mice. They are outlined in the following paragraphs.

TSPY INTERACTS WITH THE MITOTIC CYCLIN B

We have adopted an in vitro binding and pull-down assay to study the possible interaction between TSPY and cyclin B. Initially, the Xenopus cyclin A, B1 and B2 were subcloned into a GST expression vector and expression in bacterial hosts and purified from total cell extracts. The human TSPY cDNA was transcribed and translated in vitro using a TnT kit. TSPY is labeled with ^{35}S-methionine in the reaction mixture. To demonstrate an interaction, labeled TSPY proteins were reacted

independently with GST-cyclin A, B1 or B2 fusion proteins. The GST fusion and bound proteins were purified by affinity binding to glutathione-conjugated resins and analyzed by SDS-PAGE. Results from this study demonstrated that TSPY is preferentially retained by GST-cyclin B1 or B2 fusion proteins, but not by GST alone nor GST-cyclin A (Figure 1A). Similar studies with the human cyclin B confirmed that human TSPY did interact with the human cyclin B.

Previous studies demonstrated a spectrum of TSPY variant proteins in prostate cancer samples. To determine if these variant TSPY proteins do interact with the mitotic cyclin B similarly, we had subcloned the respective cDNAs of the TSPY proteins into an in vitro TnT vector. Radioactively labeled variant TSPY proteins were used in protein-binding assays with a GST- human cyclin B1 fusion protein. The results demonstrated that these variant proteins did bind to cyclin B1 and were co-precipitated with the cyclin B1 protein, but not GST alone (Figure 1B). Previous results suggested that alternative splicing events generate different transcripts deleting 92, 110 and 145 amino acids from the amino end of the predominant TSPY protein of 295 amino acids (Figure 2). These results suggested that the cyclin B binding domain is primarily located in the carboxyl end of the protein, as predicted by sequence alignment with NAP-1 and SET proteins. Although we are still uncertain on the functions of the different domains of the TSPY protein, deletions of portion(s) of its amino end might suggest a loss of certain function, assigned to this portion of the molecule while the cyclin B binding function is maintained in these shortened variants. It will be extremely interesting to define the functions of the different domains of TSPY and to evaluate what effects they may exert when they are deleted from the respective proteins.

TSPY PROMOTER AND ANDROGEN REGULATION

To evaluate the regulation of TSPY gene in prostatic cells, a cell transfection and promoter analysis strategy was implemented. Various portions of the human TSPY gene promoter were subcloned in a luciferase expression vector, such that the TSPY promoter directly regulates the reporter gene (Figure 3A). The expression of the luciferase was then assayed in a cell transfection assay. A variety of host cells were used in these studies. They included prostatic cells, LNCaP (androgen responsive) and PC3 (androgen non-responsive), GC1spg (sprmatogonia-like) and GC2spd (spermatocyte-like), COS7, HeLa and NIH3T3 cells. Preliminary results from these studies suggested that only 160-bp upstream of the transcription start-site of the TSPY gene was sufficient in directing the expression of the luciferase gene in all cell types (e.g. Figure 3B). However, a bimodal expression pattern was observed in GC1spg, GC2spd and PC3 cells (Figure 3C, D, and E respectively). When additional upstream sequences (upto –2.4 kb) were included in the promoter, a reduction of the reporter activities was observed, suggesting that a repressor-like regulatory element(s) was present upstream of the core promoter (at –160 bp). Such expression pattern was absent in the HeLa female cells (Figure 3F). Since GC1spg and GC2spd and PC3 were derived from the testicular spermatogonial, spermatocyte and prostatic cells respectively, the bimodal regulation of the TSPY promoter in these cells suggested that they might resemble a regulatory mechanism in these male-specific cells, as compared to the more generic cells, such as HeLa cells.

TSPY expression was stimulated by androgen in the responsive cell line, LNCaP while its expression was not affected by the male hormone in the non-responsive cell lines, PC3 and COS7, even in the presence of a transfected androgen receptor gene. It will be interesting to determine the mechanism(s) by which TSPY is regulated by the male hormone that has been demonstrated to be important in the etiology of prostate cancer (10).

TRASNGENIC MOUSE STUDIES ON TSPY GENE

Transgenic mouse studies are important components of the project. When targeted to prostate-specific expression, these studies can confirm the roles of the Y chromosome genes in prostatic physiology and diseases. For the past couple of years, we had implemented a tet-off transgene regulation system that allowed a manipulated expression or repression of the transgene in the host animals (11). This system constituted two components, a transactivator and a responder, by which the responder (target) gene could be regulated in bi-transgenic animals. We had successfully constructed various prostate-specific transactivator lines and responder lines. Currently, we are in the process of identifying the best combination of transactivator and responder lines for these experiments. Unfortunately, our progress had suffered somewhat with the unexpected departure of a key worker, Dr. Xing Xing Liu, in the early part of Year 3. Hence, this portion of the research was performed by the second postdoctoral fellow, Dr. Tatsuo Kido, who was also responsible for the protein and promoter work. To accomplish this and other aspects of the project, we had requested and been granted a no-cost extension of this project for 12 months.

We had also generated transgenic mice harboring the native TSPY gene. Three transgenic founders were obtained. Two of the transgenic founders showed extreme abnormalities in their stature (much smaller than non-transgenic littermates) and died within two months after birth. The third founder seemed to be mosaic animal and never passed on his TSPY transgene to its offsprings. Although inconclusive in nature, these results suggested that abnormal TSPY expression might be detrimental to the health/development of the transgenic animals. Hence, a manipulated expression of the TSPY transgene, e.g. using the tet-off system, might be essential for these experiments.

FUTURE DIRECTIONS

For the no-cost extension period, we hope to achieve additional results in two specific areas. First, we plan to finish our transgenic mouse studies using the tet-off system directly expressing the TSPY gene in the prostates of transgenic mice. So far, using the same regulatory system, we demonstrated that cells over-expressing TSPY formed tumors faster than those repressing the TSPY gene in athymic nude mice. We anticipate that over-expression of TSPY specifically in the prostates of the hosts might have similar effects.

The second goal for this period is to define the cyclin B binding domain of the TSPY protein. As discussed above, variant proteins were observed in the prostate cancer samples. Some of them had deleted a major portion of the amino terminus portion of the predominant species. If the deleted portion harbors a major regulatory function, then these shortened versions of the TSPY protein might still bind to the mitotic cyclin B, but fail to perform the function(s) ascribed to the deleted amino domain. Hence, we plan to continue and to include site-directed mutagenesis techniques in the binding studies. Eventually, such in vitro studies will be significant in defining the functions of the TSPY variants in future transgenic mouse studies.

KEY RESEARCH ACCOMPLISHMENTS

- Identify TSPY to be a strong candidate gene involved in prostate cancer
- Demonstrate the binding of TSPY protein to the mitotic cyclin B

- Demonstrate the expression of the shortened version of TSPY proteins
- Demonstrate a complex regulatory mechanism at the promoter of TSPY gene
- Demonstrate the potential role of androgen in the regulation of TSPY gene expression
- Demonstrate an aberrant expression of TSPY may be detrimental for normal development

REPORTABLE OUTCOMES

- Demonstration of a preferential elevated expression of TSPY in human prostate cancer and aberrant splicing of TSPY transcripts (and encoded proteins) in prostate cancer. These results raise the possibility that these variant/abnormal TSPY proteins may be involved in ocogenic activities in humans.

  Lau Y-FC, Lau HW and Kömüves LG (2003). Expression pattern of a gonadoblastoma candidate gene suggests a role of the Y chromosome in prostate cancer. Cytogen Genome Research, in press.

- Establishing the preliminary evidence for TSPY gene as a cell cycle regulator and promoter of cell proliferation in cultured cells and tumor formation in animals.

  Liu XX and Lau Y-FC (2003). Over-expression of the Y-chromosome gene, TSPY, potentiates cell proliferative activities in vitro and in vivo. In preparation.

- Defining the cyclin B binding domains for TSPY. This study establishes the probable mechanism of cell cycle regulation by TSPY.

  Li YM, Kido T and Lau Y-FC (2003). TSPY domain important for binding to the mitotic cyclin B, a possible mechanism of cell cycle regulation by the Y chromosome. In preparation.

CONCLUSION

The initial rationale for the present project is that the Y chromosome is a male-specific chromosome harboring genes important for the physiology of men. Any mutations or abnormal expression of one of several of these genes might affect the functions of male-specific organs, leading to diseased states, such as infertility and cancer. Through the support of the present grant award, we have systematically examined the expression of 31 relevant genes on this chromosome in prostate cancer samples and have identified the TSPY gene to be a cardinal candidate gene involved in male-specific cancer. Further studies suggest that over-expression of TSPY potentiates cell proliferation and tumor formation in nude mice. Our efforts in the past year demonstrate a variation in RNA processing of the TSPY transcripts, resulting in shortened versions of TSPY protein. Protein binding assays show that TSPY binds to the mitotic cyclin B, probably through its NAP-1/SET homologous domain. These findings substantiate the notion that TSPY participate in cell cycle regulation. Its interaction with the cyclin B may play a key mechanism for it to participate in such cell proliferation process.

SO WHAT

Uncontrolled cell proliferation is a key characteristic of tumor growth. The elevated levels of TSPY expression in prostate cancer and its potentiation of cell proliferation suggest that this Y chromosome gene may be involved in cell cycle regulation. The possible involvement of the TSPY gene in prostatic oncogenesis suggests that the Y chromosome may be involved in prostate cancer

development or progression. Understanding its mechanism contributing to oncogenesis should be significant in establishing the etiology and designing therapeutic strategies for this significant disease in men.

REFERENCES

1. Lau, Y-FC (1999). Gonadoblastoma, testicular and prostate cancers, and the TSPY gene. Am J Hum Genet, 64:921-927.

2. Lau Y-FC and Zhang J (2000). Expression analysis of thirty-one Y chromosome genes in human prostate cancer. Mol Carcin 27:308-321.

3. Lau Y-FC, Chou PM, Iezzoni JC, Alonzo JA and Komuves LG (2001). Expression of a candidate gene for the gonadoblastoma locus in gonadoblastoma and testicular seminoma. Cytogenetics and Cell Genetics, 91:160-164 (2000).

4. Schnieders, F., Dork, T., Arnemann, J., Vogel, T., Werner, M., and Schmidtke, J., Testis-specific protein, Y-encoded (TSPY) expression in testicular tissues. Hum Mol Genet, 1996. 5:1801-7.

5. Liu XX and Lau Y-FC (2001). Over-expression of the TSPY gene on the Y chromosome potentiates cell proliferation in vitro and in vivo. Am J Hum Genet 69 (supppl):245.

6. von Lindern, M., van Baal, S., Wiegant, J., Raap, A., Hagemeijer, A., and Grosveld, G., CAN, a putative oncogene associated with myeloid leukemogenesis, may be activated by fusion of its 3' half to different genes: Characterization of the SET gene. Mol Cell Biol, 1992. 12:3346-55.

7. Nagata, K., Kawase, H., Handa, H., Yano, K., Yamasaki, M., Ishimi, Y., Okuda, A., et al., Replication factor encoded by a putative oncogene, SET, associated with myeloid leukemogenesis. Proc Natl Acad Sci U S A, 1995. 92:4279-83.

8. Estanyol, J.M., Jaumot, M., Casanovas, O., Rodriguez-Vilarrupla, A., Agell, N., and Bachs, O., The protein SET regulates the inhibitory effect of p21(cip1) on cyclin E-cyclin-dependent kinase 2 activity. J Biol Chem, 1999. 274:33161-5.

9. Ozbun, L.L., You, L., Kiang, S., Angdisen, J., Martinez, A., and Jakowlew, S.B., Identification of differentially expressed nucleolar TFG-beta1 target (DENTT) in human lung cancer cells that is a new member of the TSPY/SET/NAP-1 superfamily. Genomics, 2001. 73:179-93.

10. Kasper S, Sheppard PC, Yan Y, Pettigrew N, Borowsky AD, Prins GS, Dodd JG, Duckworth ML and Matusik RJ (1998). Development, progression, and androgen-dependence of prostate tumors in probasin-large T antigen transgenic mice: a model for prostate cancer. Lab Invest 78:319-333.

11. Gossen M, Freundlieb S, Bender G, Muller G, Hillen W and Bujard H (1995). Transcriptional activation by tetracyclines in mammalian cells. Science 268:1766-1769.

APPENDIX COVER SHEET

FIGURES 1-3

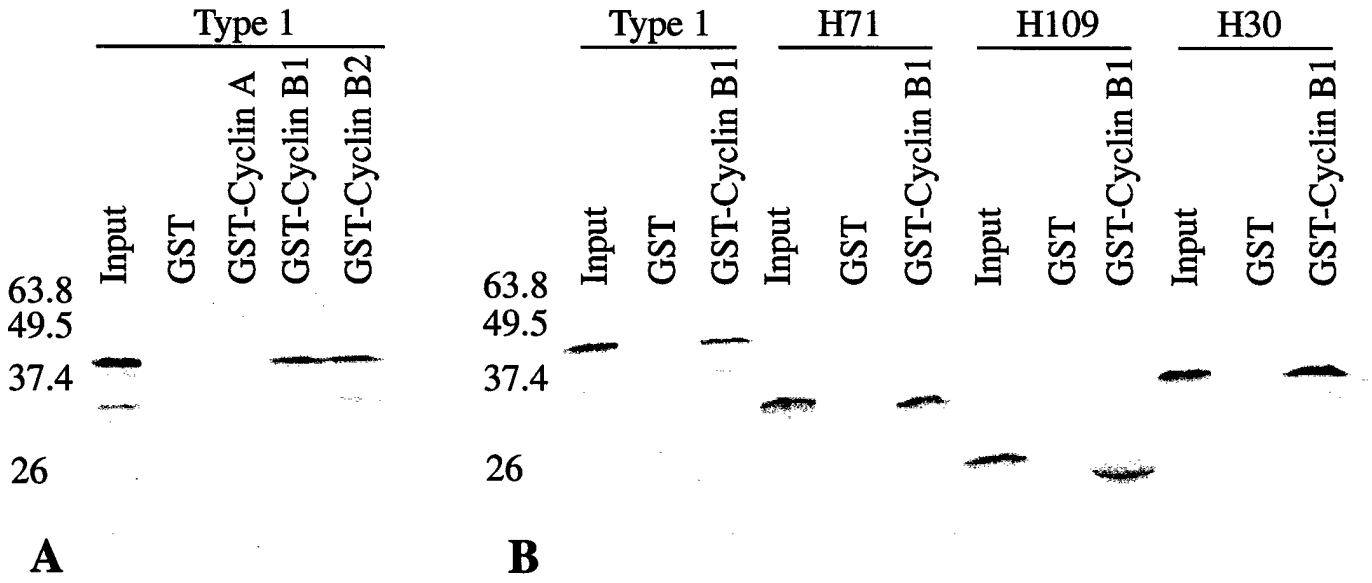

Figure 1. GST or GST-cyclin fusion proteins were induced in BL21(DE3) bacterial host by 1mM IPTG, then purified with affinity chromatography using glutathione sepharose. TSPY variants were synthesized by coupled transcription and translation reaction in the presence of ^{35}S-methinoine. GST-pulldown binding assay was performed with 40% TnT labelled products were used for each binding reaction at 4°C for 3 hours, washed 3 X with binding buffer. The bound proteins were then analyzed by SDS-PAGE autoradiography. **A.** Type 1 labelled-TSPY protein as a probe. The baits were cyclin proteins from *Xenopus*. **B.** Type 1, H71, H109, H30 were used as labeled TSPY probes. GST-human cyclin B1 fusion protein was used as a bait for the pull-down assays. Size markers are in kDa.

```
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Type 2    1 MRPEGSLTYRVPERLRQGFCGVGRAAQALVCASAKEGTAFRMEAVQEGAA  50
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  H104    1 MRPEGSLTYRVPERLRQGFCGVGRAAQAL----------------------  29
  H109    1 MRPEGSLTYRVPERLRQGFCGVGRAAQAL----------------------  29
  H106    1 MRPEGSLTYRVPERLRQGFCGVGRAAQAL----------------------  29
   H80    1 MRPEGSLTYRVPERLRQGFCGVGRAAQALVCASAKEGTAFRMEAVQEGAA  50
   H30    1 MRPEGSLTYRVPERLRQGFCGVGRAAQALVCASAKEGTAFRMEAVQEGAA  50
   H51    1 MRPEGSLTYRVPERLRQGFCGVGRAAQAL----------------------  29

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Type 3   51 GVESEQAALGEEAVLLLDDIMAEVEVVAEVEVVAEEEGLVERREEAQPRQ 100
   H71   29 ---------------------------------------------------  29
  H104   29 ---------------------------------------------------  29
  H109   29 ---------------------------------------------------  29
  H106   29 ---------------------------------------------------  29
   H80   29 GVESEQAALGEEAVLLLDDIMAEVEVVAEVEVVAEEEGLVERREEAQRAQ 100
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  H104   29 ----------------------------------------REKMERRRKPH  40
  H109   29 ---------------------------------------------------  29
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   H80  101 QAVPGPGPMTPESALEELLAVQVELEPVNAQARKAFSRQREKMERRRKPQ 150
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   H80  251 QRDPAPFPQKLLSL                                     265
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   H51  158 AYRRRHHNSSLNFFNWFSDHNFAGSNKIAEVSPHWETN             195

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Type 2  295 N               295
Type 3  301 EEGTETSGDSQLLSN 315
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  H109  156 EEGTETSGDSQLLSN 170
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Figure 2. TSPY Variant Proteins

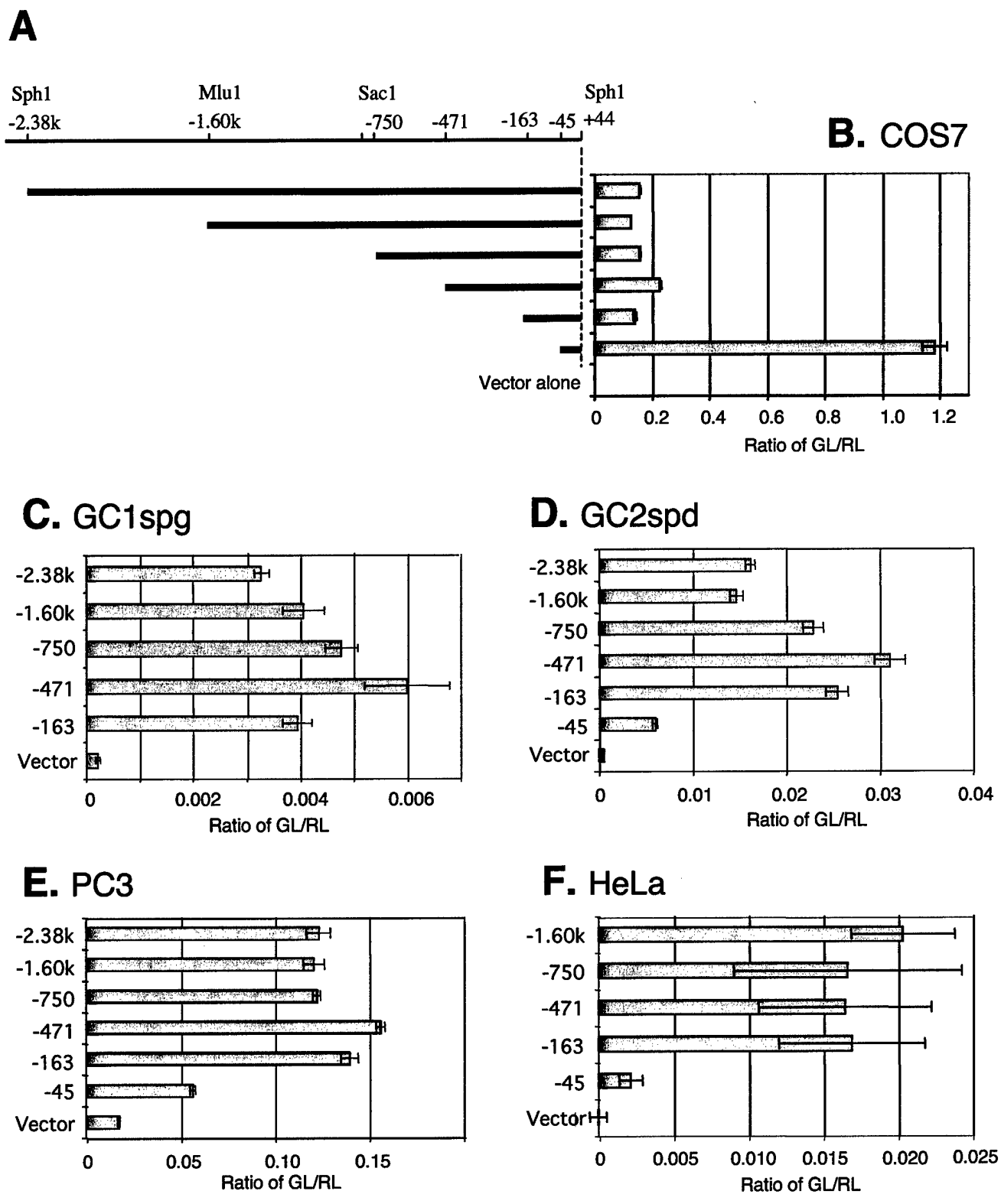

Figure 3. 0.3μg of pGL2-TSPY promoter or pGL2-basic was transfected into cultured cells with 15ng of pRL-TK (24 well plate). Transfection was performed by Fugene6 (Roche). After transfection, cells were incubated for 2 days. The promoter activity was measured by a dual luciferase assay kit (Promega). Values mean the ratio of tested promoter activity (GL)/internal control activity (RL).