Supplementary Information to Grandori et al: "The Werner syndrome protein limits MYC-induced senescence". (Genes and Development 2003 *in press*)

Abstract

The MYC oncoprotein is a transcription factor that coordinates cell growth and division. MYC over-expression exacerbates genomic instability and sensitizes cells to apoptotic stimuli. Here we demonstrate that MYC directly stimulates transcription of the human Werner syndrome gene, *WRN*, which encodes a conserved RecQ helicase. Loss-of-function mutations in *WRN* lead to genomic instability, an elevated cancer risk and premature cellular senescence. The over-expression of MYC in WRN syndrome fibroblasts or after WRN depletion from control fibroblasts led to rapid cellular senescence that could not be suppressed by *hTERT* expression. We propose that *WRN* upregulation by MYC may promote MYC-driven tumorigenesis by preventing cellular senescence.

Supplementary Figure 1 and Table I

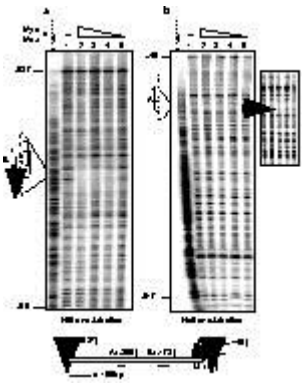
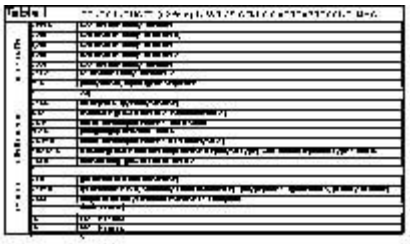


Figure 1. *In vitro* DNAseI footprint of the *WRN* promoter with purified MYC-MAX heterodimers. The *WRN* promoter (Genbank # AB003173[5]) cloned in pGL3[4] was labeled either at a HindIII site (a) located in the pGL3 vector (at a distance of ~40 bp from the transcription start site) or at the NotI site (b) present in the WRN promoter (at -537 bp), then cut with either NotI or HindIII to isolate end labeled fragments, respectively. DNA was then incubated with MYC-MAX heterodimers containing 120 ng of C92-MYC and 30 ng of MAX (lane 2) and serial dilution of 1:2.5 (lanes 3, 4 and 5). After 30 min at R.T. the samples were transferred on ice and treated with DNAseI. DNA was extracted and resolved on a denaturing 5% urea-acrylamide gel. The gel was fixed and exposed on a Phospho-Imager (in order to visualize the footprint the GA ladder was overexposed in the pictures shown). An enlargement of the protected region of site A is shown to the right of the b panel; due to the distance of the A site from the labeled end the resolution is poor, however, the results were reproducible. The arrow along the protected DNA sites indicates the direction of transcription.



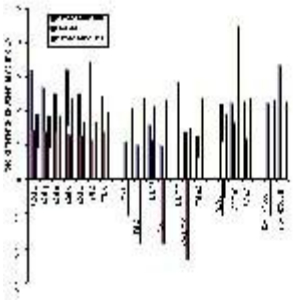


Table I. List of a selected set of genes increased by Myc (>2fold) in at least one of the WRN$^{-/-}$ cells but not in the control fibroblasts. Only a subset of the increased genes is shown in support of the phenotypic characterization of the WRN$^{-/-}$ cells after transduction with *c-myc* expressing retroviruses. In fact, among the induced genes several encode metalloproteases, previously described as characteristically increased in senescent cells[3]. The comparison included RNA prepared from *hTERT*$^{+}$,WRN$^{-/-}$ cells (AG00780 and AG03141) infected with *c-myc* retrovirus (LmycSN) or empty vector (LN) or from normal fibroblasts also with or without Myc. All samples were collected at passage 3 when the WRN cells indicated morphological changes of senescence. Each RNA pair Myc and LN was used to generate labeled cDNA with Cy3 and Cy5 respectively, and hybridized to 18,000 spotted human cDNA array, produced by the Fred Hutchinson Array Facility. The fluorescence intensity was normalized by local regression analysis following the ëlowessí scatter plot smoother[6] using f = 67% and Splus software (f is the fraction of data used for smoothing each point). The fold differences in fluorescence intensity between Myc and LN for the genes shown in Table I are shown below.

Supplementary Material and Methods

Northern analysis and RT-PCR. Northern analysis of *WRN* mRNA expression was performed with polyA+ RNA using standard techniques and a full length *WRN* cDNA probe[2]. Northern controls included *Myc-ER*, endogenous *c-myc*, *actin*, or *max* (see Figure 1). A Phospho-Imager was used to quantify steady state mRNA levels. RT-PCR primers for *h-TERT* were: 5-CGAGCTGCTCAGGTCTTTCTTTTATG-3 and 5-CCACGACGTAGTCCATGTTCACAATC-3. Primers for the ribosomal protein 36B4, used as a control, were: 5-TGCCAGTGTCTGTCTGCAGA-3 and 5-ACAAAGGCAGATGGATCAGC-3.

Chromatin cross-linking and immunoprecipitations (ChIP assay). The basic ChIP protocol and quantification was performed has described[1].The following modifications were made: 1.5-3.3 x 10^8 fixed cells were sonicated in 6 ml SDS buffer. The lysate was diluted with 3 ml of Triton Dilution Buffer (100 mM Tris pH 8.6, 100 mM NaCl, 5 mM EDTA, 5% Triton X-100). MYC and control IPs were performed using 9 ml of lysate and either 50 µg polyclonal anti-MYC antibody (N262, Santa Cruz Cat. #SC764) or 500 µl blocked protein A beads (50% slurry protein A-sepharose (Amersham). PCR was performed with 4 µl of DNA and 800 nM primers diluted to

a final volume of 20 µl in SYBR Green Reaction Mix (Perkin Elmer). Product accumulation was monitored by real-time PCR using a GeneAmp 5700 Sequence Detector (Perkin Elmer). PCR primers for non-target E-box containing genes were, for control I (ZNF127-Xp ; Genbank # U41315): 5-CCAAATCTGTAAGCCTGCTCAAC-3 and 5-GAAGGGCAGGAATGATTACTGC-3 and for control II (GOS2; Genbank # M72885): 5-ATCCAAGCGTAACCTGTTCTGTC-3 and 5-CAACCTTCTTACTGGTGTCAGCG-3. PCR primers for the *WRN* promoter (Genbank # #AB003173) A site were: 5-CGTCTCCGCTCTTCCTCAGT-3 and 5-TTCGCACTCTCCGCTGC-3 and for the *WRN* promoter B site: 5-CACCGGGATGCAGGACTCTA-3 and 5-CCACCACAGCCCATCCA-3. Due to their proximity the A and B sites are not individually identified in these assay.

Western analysis. Western analyses were performed using PBS-washed cell pellets that had been lysed in RIPA buffer or directly in SDS sample buffer prior to 3 cycles of sonication for 10 sec. Protein samples were normalized by protein concentration or cell number prior to gel electrophoresis. Primary antibodies are specified in the Figure legends and were used at dilutions recommended by the manufacturers.

References

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