

Adaptive methylation regulation of *p53* pathway in sympatric speciation of blind mole rats, *Spalax*

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Epigenetic modifications play significant roles in adaptive evolution. The tumor suppressor *p53*, well known for controlling cell fate and maintaining genomic stability, is much less known as a master gene in environmental adaptation involving methylation modifications. The blind subterranean mole rat *Spalax eherenbergi* superspecies in Israel consists of four species that speciated peripatrically. Remarkably, the northern Galilee species *Spalax galili* ($2n = 52$) underwent adaptive ecological sympatric speciation, caused by the sharply divergent chalk and basalt ecologies. This was demonstrated by mitochondrial and nuclear genomic evidence. Here we show that the expression patterns of the *p53* regulatory pathway diversified between the abutting sympatric populations of *S. galili* in sharply divergent chalk–basalt ecologies. We identified higher methylation on several sites of the *p53* promoter in the population living in chalk soil (chalk population). Site mutagenesis showed that methylation on these sites linked to the transcriptional repression of *p53* involving Cut-Like Homeobox 1 (*Cux1*), paired box 4 (*Pax 4*), *Pax 6*, and activator protein 1 (*AP-1*). Diverse expression levels of *p53* between the incipiently sympatrically speciating chalk–basalt abutting populations of *S. galili* selectively affected cell-cycle arrest but not apoptosis. We hypothesize that methylation modification of *p53* has adaptively shifted in supervising its target genes during sympatric speciation of *S. galili* to cope with the contrasting environmental stresses of the abutting divergent chalk–basalt ecologies.

evolution | speciation | ecological stress and adaptation | epigenetics

Sympatric speciation without geographic isolation is becoming more convincing with the accumulation of supportive genetic evidence (1–3). As we previously proposed (1, 3), sympatric speciation occurs during the adaptive evolution of the Israeli subterranean blind mole rat *Spalax galili* ($2n = 52$) living in the Galilee in northern Israel. Genetic and physiological differences were observed from two abutting populations of *S. galili* living in sharply divergent soil types (chalk and basalt) including population-specific haplotype mitochondrial (mt) DNA pattern, population-unique SNPs of genome, different activity patterns, and distinct oxygen consumption (1, 3). This is the first demonstration, to our knowledge, of sympatric speciation in subterranean mammals, certainly in the genus *Spalax*, which usually speciates peripatrically or allopatrically (4).

The tumor suppressor gene *p53* has been underappreciated for its roles in environmental adaptation. As a sensor of stresses including genotoxic stress and hypoxia (5, 6), *p53* is also a master gene of diversity during adaptive evolution (7–11), although this perspective is less studied than its role in cancer suppression. Modifications of *p53* in different levels during adaptive evolution confer the animal's selective advantage by regulating adaptive cell fates. Our recent study demonstrated that variation of *p53* is important for adapting to a variety of environmental stresses, including hypoxia, temperature, and pH changes (12).

Spalax is well adapted to hypoxia due to its underground habitat (4, 13–15) (see full list of publications: evolution.haifa.ac.il/index.

php/28-people/publications/152-publication-nevo-spalax). Thus, *Spalax* is an excellent model to investigate the correlation between gene variations and environmental adaptation. Our previous study revealed an important mutation on the codon 172 of *Spalax*'s *p53*, which reduced its ability to activate apoptotic target genes (9). This mechanism is believed to contribute to *Spalax*'s hypoxia adaptation by escaping from hypoxia-induced apoptosis (9). Recently, we sequenced and analyzed the genome and transcriptome from the *S. galili* population living in basalt soil (basalt population). Transcriptome analysis of the *p53* signaling pathway showed that eight of the *p53* target genes were differentially regulated by hypoxia in *S. galili* and rat, supporting the adaptive roles of *S. galili*'s *p53* pathway (16).

Although there is no geographical barrier, the two populations of *S. galili* living in the two abutting soils adopt completely different habitats, in which the chalk soil is drier and hotter especially in summer and the basalt soil is more humid and hypoxic during winter floods. Moreover, only 28% of 112 plant species are shared between the two abutting soils (1), suggesting very different diet structures of the two populations.

In addition to gene mutations, heritable epigenetic changes occur more frequently with paramount importance for adaptive evolution (17–19). We report here that the two contiguous

Significance

p53 has been shown to play important roles in environmental adaptive evolution. Here we show that *p53* and its target genes express differentially between two abutting populations of the blind mole rat *Spalax galili* during its sympatric speciation caused by sharply divergent abutting ecologies of chalk and basalt. Remarkably, the differential expression of *p53* is due to differing methylation on sites –1446, –1204, and –1086 of the *p53* promoter, which plays a key role in regulating the binding of several transcription factors including Cut-Like Homeobox 1, paired box 4 (*Pax 4*), *Pax 6*, and activator protein 1. Different expressions of *S. galili p53* selectively changed adaptive cell-cycle arrest. This article provides evidence supporting the sympatric speciation of *S. galili*, demonstrating the importance of epigenetic modifications in adaptive evolution.

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. [KP799008](https://www.ncbi.nlm.nih.gov/nuclseq/KP799008) (*S. galili p53* CDS) and [KP799009](https://www.ncbi.nlm.nih.gov/nuclseq/KP799009) (*S. galili p53* promoter)].

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populations of the blind mole rat *S. galili* living in contrasting, but abutting, chalk and basalt soils (henceforward, chalk mole rat population and basalt mole rat population, respectively), adapting to distinct degrees of hypoxia, hypercapnia, and different diet spectra, display divergent expressions of the *p53* regulatory pathway, due to different methylation modifications. The results provide important evidence supporting the significant adaptive roles of epigenetic changes of *p53* in environmental adaptation during sympatric speciation.

Results

Identical *p53* Sequences of Two Populations of *S. galili*. The coding sequence (CDS) and a 2-kb upstream promoter region of *p53* of *S. galili* from both *S. galili* abutting soil populations were cloned and sequenced (GenBank accession nos. KP799008 for the CDS and KP799009 for the promoter). A 203-bp fragment was predicted as a 5' untranslated region (UTR) by comparing the southern species *Spalax judaei* *p53* mRNA sequence (GenBank accession no. AJ783406) (9) with the genomic sequence of *S. galili* (16), aligned with human *p53* (Ensembl transcript ID ENST00000269305). Alignment analysis revealed identical nucleotide sequences of *p53* CDS and the 2-kb upstream regulatory region including the 5' UTR of the two soil mole rat populations (Fig. S14). Comparing *S. galili* *p53* with its sibling species *S. judaei* ($2n = 60$) showed that they share the same protein sequence, both carrying an R172K mutation, although there are two synonymous varied bases at positions 495 and 690 (Fig. S1B).

Divergent Expressions of *p53* and Its Supervised Genes in Tissues. To compare the expression profiles of the *S. galili* *p53* regulatory network, quantitative real-time PCR (qPCR) was performed to test the expression patterns of the *p53* and dozens of its supervised genes from the brain, liver, lung, kidney, and spleen of *S. galili* from both soil types' mole rat populations (results presented are for six animals of each population). The downstream *p53* target genes selected here include *Erc5*, *Mlh1*, *Polk*, *Msh2*, *Rrm2b*, *Xpc*, *Mgmt*, *Ddb2*, *Tigar*, *Sco2*, *Gls2*, *Gamt*, *Apaf1*, *Bax*, *Bcl2*, *Igf1p3*, *Puma*, *P21*, *Pten*, *Mdm2*, *Bai1*, and *Tsp1*. Their protein products cover the functions of DNA repair, metabolism, apoptosis, cell-cycle arrest, and anti-angiogenesis (20). The mRNA expression of *p53* showed different patterns in various tissues. The mRNA expression level of *p53* in the lung from the chalk mole rat population is twofold higher than that of the basalt mole rat population (Fig. 1). Out of the 22 selected *p53* target genes, *Erc5*, *Rrm2b*, *Bcl2*, *p21*, and *Bai1* showed significantly increased expression levels in the chalk population than those in the basalt population (Fig. 1). None of the genes were observed expressing lower levels in chalk than in the basalt population. Several cell-cycle genes expressed higher levels in chalk than in the basalt population in liver (Fig. S2). In kidney, these genes' expression in the chalk population was generally lower than in the basalt population, although no statistical significance differences were observed due to the large SE (Fig. S2). No significant difference of the genes' expression between the two mole rat populations was observed in brain and spleen (Fig. S2).

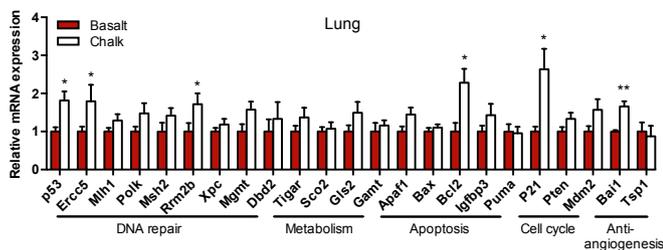


Fig. 1. *S. galili* from basalt and chalk soil types have divergent expression patterns of *p53* and its supervised genes. The mRNA expression of *p53* and its target genes in the lung of the two populations of *S. galili* living in basalt and chalk soil types is shown. Results are mean \pm SEM ($n = 6$). * $P < 0.05$, ** $P < 0.01$ (unpaired *t* test).

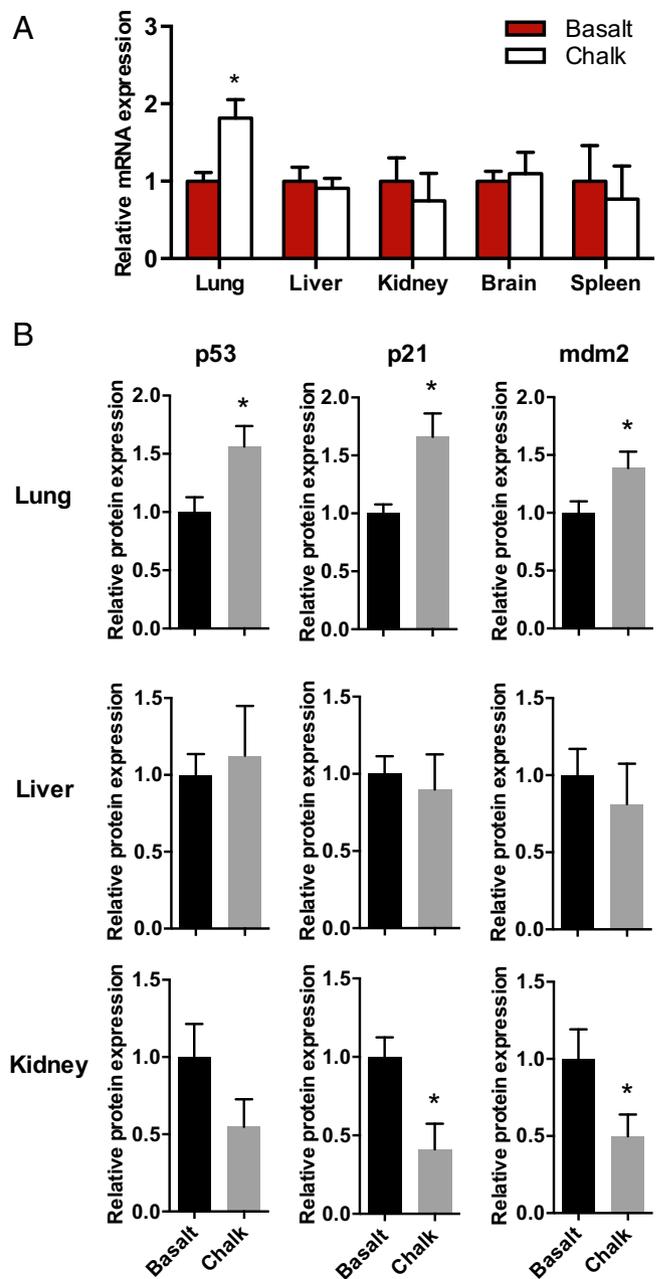


Fig. 2. Different expression patterns of *p53* mRNA and protein between chalk and basalt mole rat populations. (A) Comparing *p53* mRNA in lung, liver, kidney, brain, and spleen between chalk and basalt mole rat populations. (B) Western blot showing tissue-specific expression of *p53*, *p21*, and *mdm2* proteins in lung, liver, and kidney compared between chalk and basalt mole rat populations. Results are mean \pm SEM ($n = 6$). * $P < 0.05$ (unpaired *t* test).

Western blot was performed to show the protein expression of *p53*, *p21*, and *Mdm2*. Generally, the protein expression patterns comparing the two mole rat populations were similar with the mRNA expression, although the mRNA and protein levels of *p21* and *Mdm2* did not match in the liver (Figs. 1 and 2). This may be due to different posttranscriptional modifications. The *p53* and *p21* protein levels in the lung showed the exact same patterns with their mRNA levels (Figs. 1 and 2).

Different Methylation Status of *p53* Promoter in the Two Populations of *S. galili*. Methylation has been shown to be able to regulate gene transcription by blocking the binding of transcription

factors to the DNA double-strand (21–24). We used MethPrimer to analyze the potential CpG islands on the promoter of *S. galili* p53 (25). Two CpG islands were predicted within the 2-kb upstream promoter region of *S. galili* p53, located at -1835~1735 and -1579~-1047 (relative to the transcript start site, which was defined as +1), defined as island 1 and island 2, respectively (Fig. 3A). Besides these two islands, several CpG sites were found, although they are not methylated based on methylation analysis.

Bisulfite DNA sequencing PCR (BSP) was performed to detect the methylation status of these two CpG islands in the lung and liver of five animals from each mole rat population. Although no site was observed methylated within island 1, 25 out of 41 CpG sites within island 2 were methylated in different mole rat individuals, including 20 sites in the basalt population and 27 sites in the chalk population in the lung, as well as 14 sites in the basalt population and 20 sites in the chalk population in the liver (Fig. 3A and Fig. S3). Generally, the chalk mole rat population has higher methylation in several sites in the lung, including sites

-1446, -1204, -1195, -1092, -1086, and -1044. Remarkably, site -1044, which is on the edge of island 2, had methylation in both populations, and it was significantly higher in the chalk population than in the basalt population (Fig. 2B). Pyrosequencing in this region was performed to confirm the higher methylation on specific sites in the chalk mole rat population.

Site Methylation Contributes to Divergent Expressions of *S. galili* p53 in the Lung of the Abutting Mole Rat Populations on Chalk and Basalt.

To identify the sites responsible for the differential expression of *S. galili* p53 between the two mole rat populations, site-directed mutagenesis was performed on the *S. galili* p53 promoter luciferase reporter plasmid (namely, *S. galili* p53-Luc reporter plasmid), changing each suspected CG site to AG, which abrogates its ability to be methylated on this site. In vitro methylation was performed to induce global methylation of the plasmids. Dual-luciferase activity assay was performed after transfection of the NCI-H1299 cell line. Expression plasmids of transcription factors

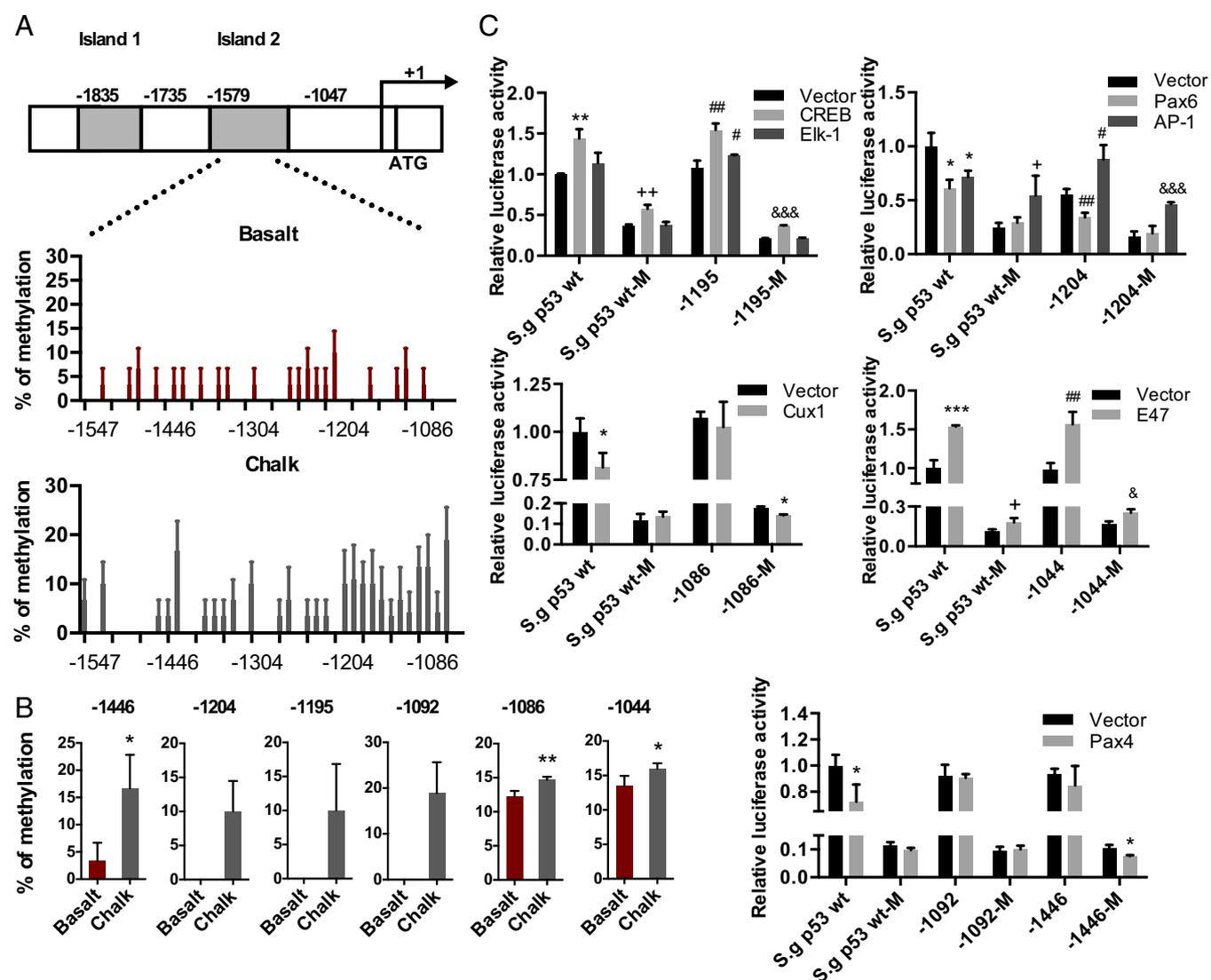


Fig. 3. The differential methylation of *S. galili* p53 promoter modulates transcription of p53 involving Cux1, Pax4, Pax6, and AP-1. (A) Profiles of methylation status on the p53 promoter of *S. galili* living in basalt and chalk soils. (B) Sites showing different degrees of methylation between the two mole rat populations. (C) Cux1, Pax4, Pax6, and AP-1 contribute to the transcriptional regulation of *S. galili* p53. Results are mean \pm SD of three independent experiments. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (unpaired t test) compared with unmethylated *S. galili* p53 groups. + $P < 0.05$ and ++ $P < 0.01$ compared with methylated *S. galili* p53 groups. # $P < 0.05$ and ## $P < 0.01$ compared with unmethylated *S. galili* mutant groups. & $P < 0.05$, && $P < 0.01$, and &&& $P < 0.001$ compared with methylated *S. galili* mutant groups.

were cotransfected with the wild-type or mutant *p53* reporter plasmids with or without in vitro methylation treatment. The selected transcription factors include cAMP response element-binding protein (CREB), Elk-1, paired box 4 (Pax4), activator protein 1 (AP-1), Cut-Like Homeobox 1 (Cux1), E47, and Pax6, which potentially bind to the suspected CpG sites based on prediction using the MATCH software. Dual-luciferase assays showed that Cux1 could repress the transcription of *p53*, and the repression was blocked by methylation of the *p53* promoter (Fig. 3C). Mutation of site -1086 rescued the repression by Cux1 (Fig. 3C), suggesting that the methylation on site -1086 blocked the binding of Cux1 to the *p53* promoter, blocking its repression. A similar pattern was observed with Pax4, which also represses *p53* transcription, and the repression was blocked by methylation. Site -1446 mutation rescued the repression of the methylated *p53* promoter by Pax4 (Fig. 3C), suggesting that site -1446 is important for the binding of Pax4 to the *p53* promoter, which could be influenced by methylation. Pax6 and AP-1 also showed different responses upon methylation of both wild-type *p53* and the site -1204 mutant (Fig. 3C). Transcription of *p53* induced by E47, CREB, or Elk-1 was not influenced by site mutation and methylation (Fig. 3C). Simultaneous mutations of CpG sites -1209/-1204/-1195 and -1122/-1110, respectively, were performed to show the integral effects of these sites. The sites -1209, -1122, and -1110 also showed higher methylation in

the chalk mole rat population, although with no statistical significance. Moreover, all these sites are located within or next to the binding region of Pax6. Based on luciferase assays, although the -1122/-1110 mutant increased the transcription of *p53*, methylation with the mutant results in an even lower expression level of *p53* compared with the wild-type *p53* promoter (Fig. S4). This change suggests that these sites contributed, at least partly, to maintaining the expression of *p53*, probably contributed by Pax6. Taken together, the results suggest that the higher expression of *p53* in the chalk mole rat population is due to the combination of methylation sites, which affects the regulation of *p53* by transcription factors including Cux1, Pax4, Pax6, and AP-1.

Divergent Expressions of *p53* from Two Abutting Mole Rat Populations Induce Different Degrees of Cell-Cycle Arrest. To assess the possible outcomes of the different *p53* expression levels between the two abutting mole rat populations, we transfected the human nonsmall cell lung carcinoma cell line NCI-H1299 and rat liver cell line BRL-3A with three levels of *S. galili* *p53* expression plasmid to mimic the different expressions of *p53* in chalk and basalt mole rat populations. NCI-H1299 cells were transfected with 2, 0.4, or 0.1 μ g of *p53* plasmids, and qPCR was performed to determine the expression of *p53* and a series of selected target genes. The expression level of *p53* decreased significantly with the decrease of

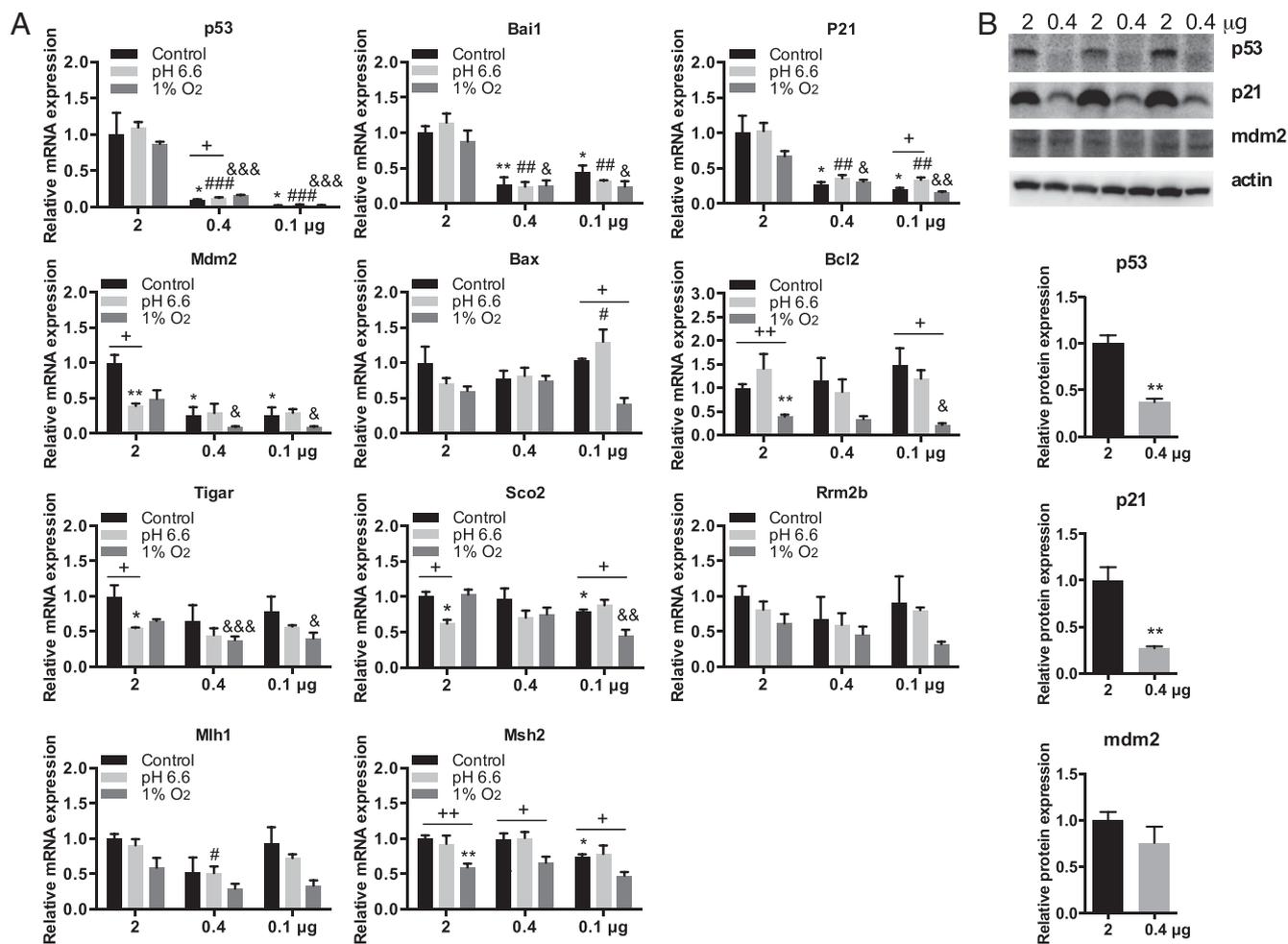


Fig. 4. (A) Selective regulation of target gene expression by different levels of *p53* under normal, acidic, and hypoxic stresses. * $P < 0.05$ and ** $P < 0.01$ compared with control groups with 2 μ g *p53* plasmid (unpaired *t* test). # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.001$ compared with 2 μ g *p53* plasmid under acidic stress. & $P < 0.05$, && $P < 0.01$, and &&& $P < 0.001$ compared with 2 μ g *p53* plasmid under hypoxia stress. + $P < 0.05$ and ++ $P < 0.01$ compared between different stresses. (B) Protein levels of *p53*, *p21*, and *mdm2* upon transfection of different levels of *S. galili* *p53*. ** $P < 0.05$ compared with 2 μ g *p53* plasmid (unpaired *t* test).

plasmid amount (Fig. 4A). Interestingly, among the 10 selected target genes, only the cell-cycle arrest genes *p21* and *Mdm2* and the antiangiogenesis gene *Bai1* decreased with the decrease of *p53*. The *p53* expression level did not influence any of the other genes involved in apoptosis, DNA repair, and metabolism (Fig. 4A). When subjected to stresses, these target genes showed different responses: Three genes were sensitive to acidic stress (pH 6.6) and two genes were sensitive to hypoxia (1% O₂) under higher expression of *p53*, whereas three genes were sensitive to hypoxia and only one gene was sensitive to acidic stress under lower expression of *p53* (Fig. 4A). Western blot was performed to confirm the different protein levels of *p53* and *p21* with different levels of transfection (Fig. 4B). Similarly, rat liver cell BRL-3A showed consistent regulating patterns between *p53* and *p21* mRNA levels even with a modest change of *p53* level (Fig. S5), although some target genes were different from human cell lines, which may be explained by the different sequence of the target gene promoters between human and rodents.

We then used flow cytometry to assess cell fates induced by different levels of *p53* under stresses. Apoptosis induced by two levels of *p53* did not show any difference, although they both decreased with a 1% O₂ challenge (Fig. 5), suggesting a protective role of *S. galili p53* under hypoxia. For cell-cycle arrest, a low level of *p53* induced a lower G1 phase arrest as determined by DNA content assay (Fig. 5). They both showed an increase in G1 arrest under hypoxia, but only the lower level of *p53* increased the G1 arrest under acidic stress (Fig. 5).

Discussion

Identical *p53* Sequence with Divergent Functional Regulation in *S. galili* Abutting Populations on Chalk Versus Basalt Soils. In the present study, we did not observe sequence differences of *p53* (neither in the promoter nor in the coding region) between the two populations of *S. galili* living in the two abutting yet distinct

soil types (chalk and basalt), representing contrasting ecologies. Remarkably, however, we found dramatically divergent expression of *p53* and genes supervised by it in the lung of the two mole rat populations (Fig. 1). The twofold higher expression of *p53* in the lung of the chalk mole rat population was accompanied with different fold changes of various target genes (e.g., *Ercc5*, *Rrm2b*, *Bcl2*, *P21*, and *Bai1*), whereas some of the genes remained unchanged. These results suggest the adaptive alterations of the *p53* regulatory network in the two abutting mole rat populations that underwent incipient sympatric speciation (1, 3). Moreover, and very importantly, the divergent expression of the *p53* pathway showed different patterns among tissues, reflecting the adaptive functional differentiation of different tissues and their physiological correlation with adaptation of respiratory, metabolic, and energy regulation.

Methylation Modification of *S. galili p53* as a Result of Adaptive Evolution. Recent studies showed that methylation changes significantly contributed to the molecular basis of evolution (17, 19). As a heritable epigenetic modification, methylation is believed to change (usually suppress) the transcription of genes by either altering the DNA structure or blocking the binding of transcriptional factors with DNA double-strand (21–24). Our results clearly showed that the methylation status differs between the chalk and basalt mole rat populations, in accordance with their diverse *p53* network expression and contrasting ecological habitats, showing the important correlation between methylation and environmental adaptation. Remarkably, we identified higher degrees of methylation on sites –1446, –1204, –1195, –1092, –1086, and –1044 of the *S. galili p53* promoter in the lung of mole rats of the chalk population, which play a role in enhancing, rather than impairing, the transcription of *p53*. Site-mutagenesis analysis showed that methylation of site –1086 blocked the binding and repression of Cux1 toward the *p53* promoter (Fig. 3C), resulting in different transcription levels of *p53* between the two abutting mole rat populations. Both CREB and E47 were shown to transactivate *S. galili p53* and were not influenced by site mutation (CREB at site –1195 and E47 at site –1044) and methylation (Fig. 3C). CREB is closely linked to the regulation of corticotrophin-releasing hormone (CRH) and its type 1 receptor (CRHR1), which was demonstrated to trigger the transcription of *p53* in rat liver and hepatic cells under hypoxia (26). The livers of chalk and basalt mole rat populations had different methylation levels on other sites—that is, –1092 and –1096. The two sites were found in the binding region of Pax4, which has been reported as a transcriptional repressor (27, 28). Transcriptional repression of *S. galili p53* by Pax6 and AP-1 (partly) was altered upon mutation on the site –1204 (Fig. 3C), suggesting the role of this site for the binding of these two transcription factors. Taken together, the different methylation levels of *S. galili p53* between the two sympatric chalk and basalt mole rat populations contribute to the different expressions of *p53* by blocking the transcriptional repressors instead of the activators of *p53*.

Selective Downstream Outcomes Reflect the Role of *p53* as a Master Regulator for Environmental Adaptation in Mole Rat Sympatric Speciation. As we observed, the twofold difference of *p53* expression between the chalk and basalt mole rat populations was accompanied with diverse downstream transcriptional outcomes, with increased cell-cycle genes *p21* and *Mdm2*, DNA repair genes *Ercc5* and *Rrm2b*, apoptosis gene *Bcl2*, and angiogenesis gene *Bai1* (Fig. 1). Mimicking the effect of altered levels of *p53* expression in human and rodent cell lines also showed a clear selective pattern toward cell-cycle arrest genes *p21* and *Mdm2* (Fig. 4A and Fig. S5). The expression of *Bcl2* was higher in the tissue of the chalk mole rat population but not in the cell line with higher transfected *p53* (Fig. 1 versus Fig. 4A). This difference may have resulted from possible sequence variations of their promoters or due to the effects of other transcription factors in the lung. The different methylation distributions on the

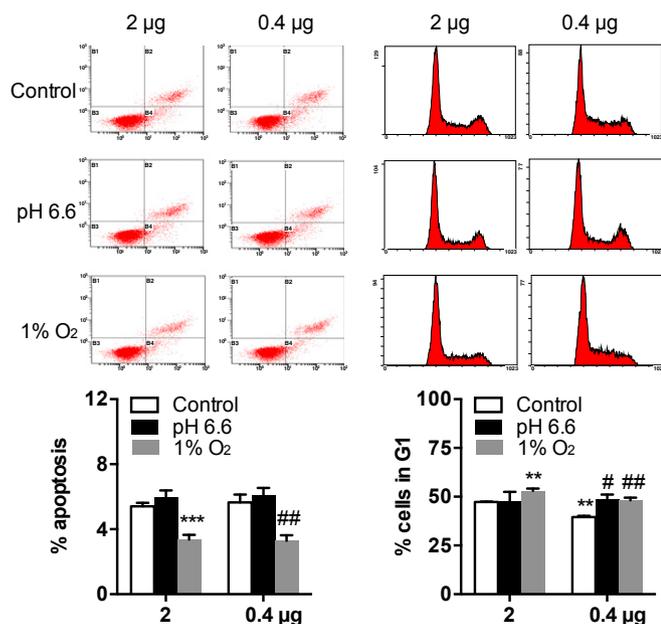


Fig. 5. The levels of *S. galili p53* are connected with divergent regulation of cell-cycle arrest. We transfected 2 or 0.4 μg of *S. galili p53* plasmid to NCI-H1299 cells, and flow cytometry reflecting *S. galili p53*-induced cell fates was shown. Apoptosis was determined as the percentage of cells with positive PE staining and negative 7-AAD staining. Cell-cycle arrest was calculated based on the DNA content. Results are mean ± SD ($n = 3$). ** $P < 0.01$ and *** $P < 0.001$ compared with the control group with 2 μg of *S. galili p53* plasmid (unpaired t test). * $P < 0.05$ and ## $P < 0.01$ compared with the control group with 0.4 μg of *S. galili p53* plasmid.

promoter itself may also be the reason for the different expression of *Bcl2* (Fig. S6).

Apoptosis induced by a higher level (in the chalk mole rat population) and a lower level (in the basalt mole rat population) of *p53* showed the same pattern, both decreased under hypoxia, suggesting a protective function of *S. galili* *p53* to adapt to the hypoxic underground burrows (Fig. 5). The H1299 cell line lacks endogenous *p53* expression, and the *S. galili* *p53* R172K mutant *p53* protein lacks the ability to induce apoptosis. Thus, the decrease of apoptosis under hypoxia with different levels of *p53* may reflect the self-protection of cells in response to hypoxia as well as the lack of proapoptotic ability of *S. galili* *p53*. In accordance with the cell-cycle arrest genes, the G1 phase arrest induced by two levels of *p53* showed differences. The lower level (basalt mole rat population) of *p53* induced a lower percentage of G1 arrest and was more sensitive to acidic stress (Fig. 5). Cell-cycle arrest is associated with energy storage. The different cell-cycle arrests in the present study support the adaptation to different oxygen consumptions and resting metabolic rates between the two abutting soil mole rat populations, which was probably due to the different degrees of hypoxia and food accessibility between the two abutting soil types, with severer hypoxia in basalt especially during winter floods (1). The pH of two soil types differs greatly, with pH 7.8–8.0 in chalk and pH 7.0–7.4 in basalt (29–31), potentially leading to different buffering ability against the high ambient CO₂ and hence hypercapnia. The more sensitive lower level of *p53* (basalt mole rat population) to acidic stress-induced cell-cycle arrest (Fig. 5) demonstrated the regulatory adaptation of *S. galili* *p53* to the different pHs in the two soil mole rat sympatrically divergent populations.

In summary, the sharply divergent chalk–basalt ecologies were the cause for the sympatric speciation of *S. galili* involving multiple adaptive complexes (1, 3). Remarkably, one of these adaptive complexes was that of the *p53* pathway, which is sensitive to differential ecological stresses, as discovered in the present work. Hence the *p53* pathway contributed remarkably to the adaptive ecological sympatric speciation of *S. galili* colonizing the new basalt

habitat some 0.2–0.4 million years ago. We showed in this study adaptive genetic and epigenetic regulatory patterns in *p53* and its supervised target genes related to cell fate under differential hypoxia and metabolism between the abutting chalk and basalt mole rat populations, and associated with adaptive ecological sympatric speciation, with restricted gene flow (1, 3) in the blind mole rat *S. galili* ($2n = 52$) in Israel. *p53* and its regulatory gene network proved to be of cardinal importance in the adaptation to stressful and changing divergent environments. The sensitive ecological adaptability of the *p53* regulatory network system may be beneficial for understanding evolutionary adaptation and speciation in nature. This might also be very relevant to human medicine, including cancer therapy.

Materials and Methods

The Institutional Ethics Committees of the University of Haifa and Zhejiang University approved all animal protocols. The blind mole rats, *S. galili* ($2n = 52$), were captured from the field in a defined region in the Upper Eastern Galilee Mountains. Ten individuals were collected from the Alma Pleistocene basalt plateau and 10 individuals from the Kerem Ben Zimra Senonian chalk (33°25' N, 35° 29.2' E, altitude 760 m above sea level) (1). Animals were euthanized, and tissues were stored in liquid nitrogen immediately after capture. Genomic DNA and total RNA were extracted from the liver, lung, spleen, kidney, and brain. Reverse transcription was performed to obtain the cDNA. The methods used in the present study (molecular cloning, qPCR, Western blot, plasmids, cell culture, transfection, methylation analysis, and dual-luciferase activity assay) are described in *SI Materials and Methods*.

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