Down-Regulation of a Host MicroRNA by a *Herpesvirus saimiri* Noncoding RNA

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To analyze the cell cycles of germ cells in the germinal cradles, we assessed the S-phase index, a measure of the percentage of nuclei in the S phase. Adult female medaka were exposed to BrdU for various periods of time (fig. S6A). After 24 hours, nearly 60% of the Gs cells were BrdU positive, and the rate of newly stained cells slowed over the following week such that about 75% of the Gs cells were BrdU positive. Thus, Gs cells appear to be heterogeneous. Piecewise linear regression analysis revealed two distinct populations: fast-dividing (GsF) and slow-dividing (Gss) germ cells. BrdU pulse-chase experiments also identified label-retaining germ cells among the Gs cells (fig. S6, B to D). These experiments suggest that at least 60% of Gs cells are Gsf cells. In contrast, the percentage of BrdU-positive Gcys nuclei increased from about 35% after 1 hour of BrdU labeling to nearly 60% after 24 hours. Thus, no quiescent Gcys cells were present in the germinal cradles.

We next addressed the potential functions of the Gs cells. We conducted experiments to determine whether Gs cells facilitate the recovery of Gcys cells after busulfan treatment. Three- to 4-month-old female medaka were treated with 10 ng/ml busulfan for 1 week to eliminate mitotically active Gcys cells. The number of germinal cradles with Gcys cells was markedly reduced 1 month after treatment, before recovery to the numbers observed in untreated control samples 3 months after the treatment (fig. S7). These results suggest that the Gs cells present 1 month after oogenesis were capable of continuously generating fertile eggs.

We identified and characterized ovarian cords within the germinal epithelia of medaka ovaries. These cords were composed of sox9b-expressing cells and contained mitotic nos2-expressing Gs cells in discrete structures referred to as germinal cradles. These mitotic oogonia continually gave rise to germ cells that developed in the ovary, finally resulting in fertile eggs. Thus, we conclude that the ovarian cord harbors the histological niche within the ovary where germinal cradles are formed (Fig. 3). Moreover, these cradles contain oogonia characteristic of germ line stem cells that contribute to the production of fertile eggs (Fig. 3).

The germinal cradle in the ovarian cord is reminiscent of the germarium from the Drosophila ovary (I); both promote the development of germ cells from germ line stem cells to very early diplo- tene oocytes in a unique histological compartment within the ovary. These similarities might reflect a fundamental process governing oogenesis across animal species.

To confirm that the nos2-expressing Gs cells generated fertile eggs, F1 embryos from heat-treated transgenic medaka were genotyped (fig. S8 and table S2). Some embryos possessed an un-cleaved losp region, whereas others exhibited the characteristic pattern of successful losp-mediated recombination (Fig. 2F). Embryos with the cleaved losp region were produced for 3 months after heat treatment (table S2), indicating that the population of nos2-expressing Gs cells was capable of continuously generating fertile eggs.

References and Notes
22. We thank M. Yamashita and T. Iwai for anti-medaka SYCP antibodies, T. Cerny for plasmids containing the hs promoter region, and S. Yoshida for helpful discussions. We are grateful to Y. Ichikawa and C. Kinoshita for maintaining the fish colony. This work was supported in part by Grants-in-Aid for Scientific Research on Innovative Areas, “Gamete Stem Cells” (grant 21116509) and for Young Scientists (B) (21770072) (to S.N.), and for Scientific Research on Priority Areas (B) (21730101); the National BioResource Project Medaka; and the Daiko Foundation and the Center for the Promotion of Integrated Science (EPS) of SOKENDAI (to M.T.).

Supporting Online Material
www.sciencemag.org/cgi/content/full/science.1185473/DC1
Materials and Methods
SOM Text
Figs. S1 to S9
Tables S1 and S2
References
Movies S1 to S3
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Down-Regulation of a Host MicroRNA by a Herpesvirus saimiri Noncoding RNA
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T cells transformed by Herpesvirus saimiri express seven viral U-rich noncoding RNAs of unknown function called HSURs. We noted that conserved sequences in HSURs 1 and 2 constitute potential binding sites for three host-cell microRNAs (miRNAs). Coimmunoprecipitation experiments confirmed that HSURs 1 and 2 interact with the predicted miRNAs in virally transformed T cells. The abundance of one of these miRNAs, miR-27, is dramatically lowered in transformed cells, with consequent effects on the expression of miR-27 target genes. Transient knockdown and ectopic expression of HSUR 1 demonstrate that it is necessary for the expression of genes involved in T cell transformation.

Herpesvirus saimiri (HVS) infects T cells and causes aggressive leukemias and lymphomas in New World primates (1). In transformed marmoset T cells, the most abundant HVS transcripts are seven small noncoding RNAs (ncRNAs) called HSURs (H. saimiri U-rich RNAs) (2–4). HSURs exhibit structural but little sequence similarity to cellular small nuclear RNAs (snRNAs).
HSURs are encoded by all HVS subgroups; HSURs 1 and 2 (Fig. 1A) are the most highly conserved and the only snRNAs expressed by the closely related *Herpesvirus atelis* (5). Because HSURs are dispensable for transcription in vitro (6, 7), their strong conservation suggests an in vivo role in infected monkeys. HSURs 1 and 2 contain highly conserved AU-rich 5′-end sequences (Fig. 1A and figs. S1 and S2) that are similar to AU-rich elements (AREs) found in the 3′ untranslated regions (3′UTRs) of short-lived mRNAs (8–10). HSURs 1 and 2 are responsible for the up-regulation of a handful of host proteins that are hallmarks of T-cell activation (11) and may contribute to an enhanced growth rate (7) of transformed cells.

Comparisons of HSUR 1 (fig. S1) and HSUR 2 (fig. S2) between HVS strains identified stretches of perfectly or highly conserved sequences (Fig. 1A, bold nucleotides). Bioinformatic searches then revealed complementarity between these HSUR sequences and three microRNAs (miRNAs) expressed in T cells: miR-142-3p, miR-27, and miR-16 (Fig. 1A) (12).

Coimmunoprecipitation experiments on extracts of virally transformed marmoset T cells using antibodies to Ago2 showed that HSURs 1 and 2 were selectively present as compared with control immunoprecipitates, whereas all other HSURs (Fig. 1B, lanes 3 and 5) and cellular small nuclear ribonucleoproteins (snRNPs) (fig. S3) did not detectably associate with micro-ribonucleoproteins (miRNPs). Immunoprecipitation was then performed on extracts from T-cell lines transformed with either the wild-type HVS strain A11 or a mutant deleted for HSURs 1 and 2 (7) by using antibodies to Sm proteins, which recognize both cellular and viral snRNPs (3). Figure 1C (lanes 5 and 10) reveals the association of miR-16, miR-27, and miR-142-3p, and not of the control miR-20, but only when HSURs 1 and 2 are present. Psoralen [aminomethyltrioxsalen (AMT)] crosslinking experiments (fig. S4) (13) confirmed the existence of in vivo interactions between miR-27 and HSURs.

We noticed a distinct difference in the overall level of miR-27 in the marmoset T-cell line transformed by wild-type HVS as compared with that of the mutant lacking HSURs 1 and 2 (Fig. 1C and fig. S5). The miR-27 family includes miR-27a and miR-27b, which are transcribed from different chromosomes and differ by only one nucleotide near the 3′ end. Quantitative real-time polymerase chain reaction (PCR) confirmed the higher abundance of both miR-27a and miR-27b in transformed T cells lacking HSURs 1 and 2 (Fig. 2A). Levels of miR-23a and miR-24, two miRNAs contained in the same primary transcript as miR-27a (14), were unchanged (Fig. 2A and fig. S5), which suggested posttranscriptional differences in the expression of miR-27 between these two cell lines. The abundance of the precursor miRNAs (pre-miRNAs) for miR-27a and miR-27b (fig. S6) did not differ between the two cell lines, nor did that of the passenger strand of the miR-27a duplex (Fig. 2A), which suggests that miRNA processing by Drosha and Dicer (15) is not altered.

To determine whether the difference in the abundance of miR-27 is due to a change in the stability of the mature miRNA, we designed a pulse-chase strategy using synthetic miRNA duplexes (16) in which only the guide strand was radioactively labeled. After nucleofection (the “pulse”), we monitored the miRNA remaining over time (the “chase”); Fig. 2B) in marmoset T cell lines transformed by either wild-type HVS or mutant HVS lacking HSURs 1 and 2. miR-27a was degraded more rapidly in the wild-type transformed cells, whereas no difference was observed for either miR-16, which is predicted to bind HSUR 2 (Fig. 1A), or for the control miR-20a (fig. S7).

HSURs 1 and 2 do not affect the steady-state levels of host miRNAs in virally transformed marmoset T cells, except for eight genes (8, 11) that are not predicted targets of HSUR-bound miRNAs.
miRNAs (17). We analyzed the levels of forkhead box 1 (FOXO1) protein, whose mRNA is a validated target of miR-27 (18). The difference in miR-27 abundance correlates with up-regulation of the FOXO1 protein (Fig. 2C) in the presence of HSURs 1 and 2, which suggests that these HVS ncRNAs perturb host gene expression via the miRNA pathway.

To confirm that the difference in miR-27 levels does not result from accumulated mutations in the two HVS-transformed T cell lines, we treated cells that contained wild-type HVS with chimeric oligonucleotides that effectively induce degradation of complementary nuclear RNAs (19). Knockdown of HSUR 1 but not of HSUR 2 correlated with higher levels of miR-27 (Fig. 3, A and B) and with lower levels of the miR-27 target protein, FOXO1 (Fig. 3C), which suggests that HSUR 1 is specifically involved in regulating miR-27.

Direct base-pairing between HSUR 1 and miR-27 is required to control miRNA abundance. Human Jurkat T cells were stably transfected with a plasmid containing HVS DNA that encodes all seven HSURs, including their endogenous transcription and processing signals (7). Precipitation of HSUR snRNPs with antibodies to Sm proteins confirmed their association with miR-27 in extracts of this cell line (Fig. 4B, lanes 1 to 5). In contrast, antibodies to Sm proteins did not communoprecipitate miR-27 from extracts of Jurkat T cells stably transfected with a plasmid deleted for the HSUR 1 gene (Fig. 4B, lanes 6 to 10). Likewise, mutation of the conserved miR-27 binding site in HSUR 1 [Fig. 4A, HSUR 1 mutant (H1M6)] abolished the immunoprecipitation of miR-27 (Fig. 4B, lanes 11 to 15). Furthermore, mutations in HSUR 1 that were designed to produce complementarity to miR-20a (Fig. 4A, H1m20) enabled a previously unknown interaction with miR-20 (Fig. 4B, lanes 16 to 20).

Expression of wild-type HSUR 1 alone (fig. S8) in Jurkat T cells (fig. S9) is sufficient to down-regulate the level of miR-27a as compared with transfection with the empty vector [Fig. 4C; green fluorescent protein (GFP)]. Direct interaction between HSUR 1 and miR-27 is required because cells transfected with a H1Mt that is unable to bind miR-27 (Fig. 4B) have levels of miR-27 comparable with those of cells transfected with the empty vector. Moreover, the miR-20a level was substantially lower after transfection of the HSUR 1 mutant (H1m20) that binds this miRNA (Fig. 4B). Together, these results indicate that base-pairing to an internal site in HSUR 1 is both necessary and sufficient to direct a mature miRNA into a cellular degradation pathway.

The ARE-like sequence in HSUR 1 is known to induce in vivo decay of HSUR 1 itself (9), suggesting that the ARE could be involved in the HSUR 1-dependent decay of miR-27. We transfected Jurkat T cells with a mutant HSUR 1 containing two U–G substitutions in the ARE (H1M1) that were previously shown to stabilize and raise cellular levels of HSUR 1 (9). This mutation resulted in higher levels of HSUR 1 (fig. S9) and did not alleviate but produced a more pronounced down-regulation of the abundance of miR-27 as compared with wild-type HSUR 1 (Fig. 4C), indicating that HSUR 1 directs the degradation of miRNAs by an ARE-independent mechanism.

We have demonstrated that HSUR 1 and 2 snRNPs directly bind specific host miRNPs in virally transformed T cells. Whereas the interaction of miR-27 with an internal site in HSUR 1 results in the degradation of this miRNA, the binding of miR-142-3p and miR-16 to HSURs 1 and 2 does not result in their lowered levels (Fig. 2, A and B, and fig. S5). Nonetheless, mutational alteration of its binding site in HSUR 2 indicates that the interaction with miR-16 also occurs via base-pairing (fig. S10), and it is conceivable that if this base-pairing were stronger, decay would be induced. Because HSURs are comparable in abundance with the bound miRNAs in virally transformed T cells (table S1), it seems unlikely that they could effectively compete with mRNA targets and act as miRNA sponges (20) even though down-regulating the activity of these miRNAs might be advantageous for the virus. For instance, miR-16 is reported to target cell-cycle and apoptosis regulators such as Bcl-2 and cyclins D1 and E1 (21, 22), but we do not observe differences in levels of miR-16 target proteins in the presence versus absence of HSURs 1 and 2 (fig. S11). The functional importance of the interaction.

**Fig. 2.** The presence of HSURs 1 and 2 affects miR-27a abundance, decay, and target expression. (A) Relative levels of different mature miRNAs in virally transformed marmoset T cells expressing (Wt) or lacking (Mut) HSURs 1 and 2 were determined by means of quantitative real-time PCR. (B) Pulse-chase assay assessing the decay of radioactively labeled synthetic miR-27a and miR-16. (C) Western blot analysis of FOXO1 in marmoset T cells transformed by HVS expressing (Wt) or lacking (Mut) HSURs 1 and 2.
between HSURs 1 and 2 and miR-16 and miR-142-3p requires further investigation.

It is not yet clear how down-regulation of miR-27 benefits HVS. Down-regulation of the same host miRNA has been reported for another herpesvirus, murine cytomegalovirus, upon infection of cell lines and primary macrophages apparently also at the posttranscriptional level (23). Only a few targets of miRNA-27, including the transcription factors FOXO1, RUNX1 and PAX3, have been validated (18, 24, 25). Thus, identification of additional targets of miR-27 in T cells transformed with HVS is needed, as well as elucidation of the molecular mechanism by which association with HSUR 1 leads to miR-27 decay.

References and Notes
17. Materials and methods are available as supporting material on Science Online.

MicroRNA-33 and the SREBP Host Genes Cooperate to Control Cholesterol Homeostasis

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Proper coordination of cholesterol biosynthesis and trafficking is essential to human health. The sterol regulatory element–binding proteins (SREBPs) are key transcription regulators of genes involved in cholesterol biosynthesis and uptake. We show here that microRNAs (miR-33a/b) embedded within introns of the SREBP genes target the adenosine triphosphate (ATP) binding proteins (SREBPs) are key transcription regulators of genes involved in cholesterol biosynthesis and uptake. We show here that microRNAs (miR-33a/b) embedded within introns of the SREBP genes target the adenosine triphosphate (ATP) binding cassette transporter A1 (ABCA1), an important regulator of high-density lipoprotein (HDL) synthesis and reverse cholesterol transport, for posttranscriptional repression. Antisense inhibition of miR-33 in mouse and human cell lines causes up-regulation of ABCA1 expression and increased cholesterol efflux, and injection of mice on a western-type diet with locked nucleic acid–antisense oligonucleotides results in elevated plasma HDL. Our findings indicate that miR-33 acts in concert with the SREBP host genes to control cholesterol homeostasis and suggest that miR-33 may represent a therapeutic target for ameliorating cardiometabolic diseases.

Cholesterol and other lipids play key roles in many physiological processes in metazoans, and aberrant cholesterol/lipid homeostasis has been linked to a number of diseases, including atherosclerosis, metabolic syndrome, and type II diabetes, underscoring the importance of understanding fully how cholesterol/lipid homeostasis is regulated (I, 2).
Battling the Paper Glut

THE ACADEMIC COMMUNITY CONTINUES TO BELIEVE THAT THE FORMAL SCHOLARLY PUBLISHING process separates sound research from shoddy or biased counterparts. Unfortunately, scholarly publishing may not be able to effectively fulfill its role as a gatekeeper much longer.

As soon as the “publish or perish” concept (the imperative to publish work constantly to further or sustain an academic career) surfaced in the United States in the early 1950s, academics criticized it openly as a recipe for disaster (1, 2). Nevertheless, in the early to mid-1980s, administrators in universities systematically began to use the number of articles published per year by individual faculty members as a measure of their productivity.

The shift transformed scholarly publishing. Researchers began “salami-slicing” their manuscripts in ever smaller “least publishable units” and began rushing manuscripts to publication before proper replication or evaluation of results. Multi-authored manuscripts increased, regardless of true contribution to the work. Doctoral students began to write dissertations as a series of publishable chapters, some submitted even before the defense. As a result, the quantity of articles published in scholarly journals increased on average by about 200 to 300% from the early 1980s to the late 1990s (3).

Researchers in countries such as China and India are subjected to a numbers game similar to that in the West, sometimes with the added incentive of monetary rewards for articles published in “top” journals. In 2008, China passed the United States to become the second scholarly producer (in total number of articles) after Europe.

Researchers have reacted to this publication glut by developing bibliometric indices, such as the h- and g-indexes, based on citation counts, to evaluate a researcher’s impact in their discipline. Perhaps these indexes do evaluate impact better than counting annual number of articles. However, in various ways, they also encourage researchers to publish more articles to directly inflate their own citations or to cite friends who then cite them in return.

The top journals now are flooded with numbers of manuscripts beyond most editors’ capacity to handle. Reviewers are solicited to scrutinize not just manuscripts but also research proposals and governmental reports. Yet, peer-reviewing is rarely, if ever, valued by academic institutions as a fruitful way for researchers to spend their time, so finding good reviewers has become more and more difficult.

Researchers need to fight to contain the current paper glut. The number of articles published per year should never be used, under any circumstance, as a criterion in tenure or promotion decisions, or to rank academic institutions. As the medical community proposed 25 years ago (4), researchers should never be allowed to include more than three publications per year in activity reports; in research proposals, principal investigators should cite no more than 10 papers. University administrators should consider peer-reviewing as not only legitimate, but a vitally important way for researchers to contribute to scholarship, and should reward it as such. One way to accomplish this would be a new generation of review impact indexes, based on information provided by publishers (3, 5). Effectiveness in peer-reviewing should be viewed as an essential skill to acquire for Ph.D. students, worldwide. Journals should demand that for every paper submitted, an author provide three reviews of other manuscripts. Perhaps if authors knew that their reviewing workload would increase dramatically with the number of papers they submit, they would craft fewer and better papers, ultimately benefiting all involved.

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References

India’s Courteous Creativity

IN HIS EDITORIAL “IRREVERENCE AND INDIAN science” (30 April, p. 547), R. A. Mashelkar observes that Indian science lacks adventure and a spirit of questioning established ideas. He suggests that the situation has deep roots in Indian culture and tradition.

I disagree. Creativity can and does exist in a society that values decorum over irreverence, such as India. In fact, a healthy skepticism, an ability to be introspective, and an urge to revisit and reexamine existing ideas have always been part of India’s intellectual tradition. Take, for example, literary works known as bhashyas, which are commen-

LETTERS

edited by Jennifer Sills

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Archaeology Augments Tibet’s Genetic History

T. S. SIMONSON ET AL. (“GENETIC EVIDENCE for high-altitude adaptation in Tibet,” Reports, 2 July, p. 72) and especially X. Yi et al. (“Sequencing of 50 human exomes reveals adaptation to high altitude,” Reports, 2 July, p. 75) estimate that the genetic divergence of Tibetan populations with unique high-altitude adaptations occurred as late as ~2750 years ago. We have investigated this same problem from an archaeological perspective. Our results partly support the genetic-based scenario but suggest some contradictions between the two data sets. We currently have no evidence of permanent occupations on the Qinghai-Tibet Plateau before the middle Holocene, ~7000 years before the present (yr B.P.) (1), contrary to claims of occupations as old as 30,000 yr B.P. (2, 3). Mobile foragers did exploit the Plateau margins up to 3300 m by ~15,000 yr B.P. (4). Directly dated sites documenting human presence above 4000 m are younger still, at ~11,000 to 8000 yr B.P. (1). These early sites represent intermittent, seasonal occupations by populations who most likely spent much of their time at lower elevations. Foragers may have established more permanent occupations on the Plateau margins as high as 3300 m after ~7000 yr B.P. (5–7), but these groups interacted extensively with agricultural populations in low-elevation environments.

Year-round occupation above 4000 m likely became possible only after 4000 yr B.P. with the emergence of dedicated pastoralist adaptations centered on domesticated yaks (6, 8). If the genetic traits suggested by Simonson et al. and Yi et al. evolved in response to selection on populations living exclusively above 4000 m, then the genetic divergence dates of ~2750 yr B.P. reasonably agrees with the archaeological evidence. If selection for these traits occurred among populations below 4000 m (2), where most Tibetans currently live, then more complex population dynamics are indicated. Understanding the archaeological chronology behind the peopling of the Qinghai-Tibet Plateau is critical to evaluating the tempo of selection operating on contemporary human populations.

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References


Response

WE THANK BRANTINGHAM ET AL. FOR THEIR interest in our study; we agree that both molecular and archaeological evidence should be

CORRECTIONS AND CLARIFICATIONS

News Focus: “From pigs to people: The emergence of a new superbug” by D. Ferber (27 August, p. 1010). Tara Smith’s affiliation is the University of Iowa in Iowa City.

Reports: “Down-regulation of a host microRNA by a Herpesvirus saimiri noncoding RNA” by D. Cazalla et al. (18 June, p. 1563). The third author should have been listed as Joan A. Steitz. The correction has been made in the HTML version online.

Reports: “Evolution of an expanded sex-determining locus in Volvox” by P. Ferris et al. (16 April, p. 351). The legend for figure 512 (Alternative splicing of female and male MAT3) in the Supporting Online Material should include the citation A. Kianianmomeni et al., Plant Cell 20, 2399 (2008), which reported two instances of unregulated intron retention (corresponding to E1_3.3, E3_5.4) and one instance of unregulated alternative splice site usage (corresponding to E13_16.5) in female MAT3. A revised Supporting Online Material has been posted online at www.sciencemag.org/cgi/content/full/328/5976/ 351/DC1.

Reports: “N-doping of graphene through electrothermal reactions with ammonia” by X. Wang et al. (8 May 2009, p. 768). After publication, the authors discovered that the graphene sheet (GS) sample used to take the x-ray photoelectron spectroscopy (XPS) and nanometer-scale secondary ion mass spectroscopy data in Fig. 4 was unintentionally oxidized by air from a leak that had not been detected during the experiment. The NH$_3$ annealing environment for the GS sample in Fig. 4 should be corrected to ~800 mtorr of NH$_3$ and an estimated partial pressure of oxygen of tens of millitorr.

Later, the authors found that as-made GSs annealed in NH$_3$, without any oxygen showed little n-doping within the detection limit of XPS, which is much lower than the doping level for the gas-phase–oxidized GS in Fig. 4. In a systematic study, they used XPS to observe n-doping and covalent N incorporation into the lattice of pre-oxidized GSs upon annealing in NH$_3$ [X. Li et al., J. Am. Chem. Soc. 131, 15939 (2009)]. They found that graphene oxide (with reduced oxidation and defect densities by stepwise thermal treatment) showed reduced n-doping levels upon NH$_3$ annealing [X. Li et al., J. Am. Chem. Soc. 131, 15939 (2009)], suggesting that the degree of n-doping scales with the degree of oxidation or concentration of defects in the graphene lattice.

The above findings are consistent with each other and do not change the main conclusions of the original publication—i.e., that annealing of graphene in NH$_3$ affords n-doping most likely at the edges and defect sites. The sample in Fig. 4 with unintended oxidation showed higher N signals than later samples without oxidation after similar NH$_3$ annealing, because gas-phase oxidation generated more defects and oxygen groups in the GS and increased its reactivity, allowing for large amounts of n-dopants to be incorporated into the GS. This finding is consistent with the authors’ original suggestion and also shows that a higher defect density in graphene introduced by gas-phase oxidation allows for higher n-doping.
used to understand the demographic history of the Tibetan people. Our Report focused not on the demographic history of the Tibetan population, but rather the selection acting on specific putatively adaptive mutations segregating in the Tibetan population. We included some limited demographic analyses because they helped illuminate our results regarding natural selection. The real demographic model is clearly likely to be more complex than the simple models of two populations diverging from each other. For example, Zhao et al. (1) used mitochondrial DNA to argue that late settlers of the Tibetan plateau may not have entirely replaced the original population but that a small proportion of them carry mitochondrial DNA lineages tracing back to late Paleolithic inhabitants on the plateau. If this is the case, even if the episodic variant was present in the early inhabitants of Tibet, strong selection would be needed to increase its frequency in the modern Tibetan gene pool. The understanding that the majority of the current population of the Tibetan plateau may trace their genetic ancestry back to quite recent immigrants into Tibet, even though humans have lived in Tibet for a much longer time—possibly with some continuity of culture—is important for understanding the difference between inferences based on archaeology and inferences based on genetics.

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Reference

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