which is involved in anti-angiogenic pathways, was identified in a study in which the microRNA cluster region was expressed in 293 cells and cellular gene expression was compared with that of control cells[7]. Two groups independently showed that KSHV-encoded miR-K11 has a seed sequence that exactly matches that of the cellular-encoded microRNA miR-155 and that the two microRNAs share overlapping targets[8,9]. Among these is BACH-1, a transcriptional repressor. These findings are of great interest because miR-155 has many roles, especially in B lymphocyte maturation, and is highly expressed in many B-cell lymphomas. Intriguingly, miR-155 is not expressed in the KSHV-associated primary effusion lymphomas, but miR-K11 is highly expressed.

Viral strategies

BCLAF1 joins a relatively small list of proven targets of virally encoded microRNAs (**Table 1**). As had been predicted, viral microRNAs target both viral and host genes. One of the first to be identified was in the rhesus polyomavirus SV40, in which the large T antigen, produced early in the viral life cycle, is degraded by microRNAs expressed later, leading to reduced recognition of infected cells by cytotoxic T cells[10]. More recently, microRNAs that perform the same function

have been identified in two human polyomaviruses: JC and BK (ref. 11). Human cytomegalovirus (HCMV) encodes a microRNA, miR-UL112, that has been shown in two independent studies to target multiple viral genes involved in viral replication[12], as well as a host gene (encoding major histocompatibility complex class I–related chain B (MICB)) that is critical for NK cell killing of virally infected cells[13]. Downregulation of MICB leads to reduced NK cell killing of HCMV-infected cells—another example of virally encoded microRNA modulating the host immune response. However, in this case, the interaction occurs directly with a host immune system gene rather than through the degradation of a viral antigen. Four of the HCMV microRNAs are encoded within HCMV coding regions and are directly antisense to the open reading frames of expressed transcripts, which suggests those viral genes as potential targets[14]. A similar observation has been made for miR-BART-2, one of the microRNAs encoded by EBV[1], but formal proof is still needed in both cases. Three microRNAs encoded by EBV (miR-BART 1-5p, MiR-BART-16 and miR-BART-17-5p) have been shown to target the viral latent membrane protein-1 (LMP-1), an oncogenic protein expressed in nasopharyngeal carcinoma[15].

All told, the report from Ziegelbaur et al.[6] represents an important addition to this new and growing field. The identification of a cellular target for a KSHV-encoded microRNA and a role for viral microRNAs in the fine regulation of the switch from latency to lytic replication is an important advance. More important, however, is the elegant strategy used and the promise this represents for further identification of microRNA targets and better understanding of microRNA functions in KSHV biology, viral oncology and more broadly in biological systems.

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Evaluating signatures of sex-specific processes in the human genome

Carlos D Bustamante & Sohini Ramachandran

Comparing levels of genetic variation between the X chromosome and autosomes can reveal the different demographic histories of males and females of a species. Taking this approach, two new studies report that the effective population sizes of men and women differ, but they disagree as to which sex outnumbered the other.

Evolutionary forces and social practices that differentially impact males and females can lead to divergent patterns of genetic variation among the autosomes, X chromosome, Y chromosome and the mitochondrial genome[1,2]. For example,

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populations in which a few males father a disproportionate number of offspring show low levels of Y-chromosomal genetic diversity. Likewise, societies in which females migrate to their husbands' homes after marriage (patrilocality) experience more gene flow of X chromosomes and mtDNA[3,4]. Investigating genomic signatures of sex-biased processes has relied on comparing the nonrecombining Y chromosomes and mitochondrial genomes[5], and it is hoped that the availability of genome-level data will provide a much finer-scale view of both patterns and processes[6–8]. On page 66 of the current issue, David Reich and colleagues[9] report evidence for accelerated genetic drift on

the X chromosome (that is, smaller effective population size, N_e, of females) during human migrations out of Africa, based on finding disproportionately low sequence variation and high SNP frequency differentiation of the X chromosome relative to that of the autosomes in non-African populations. Their results are in contrast to a recent paper by Michael Hammer and colleagues, who with a different study design estimated an unexpectedly high X-chromosomal diversity[10].

More men on the move?

Keinan et al.[9] compared genetic diversity among 130,000 X-chromosomal and autosomal SNPs

genotyped in individuals of Northern European, West African and East Asian ancestry[11,12]. They use a statistic Q that compares rates of genetic drift of X-linked and autosomal SNPs and has an expected value of 0.75 when the effective population size of males and females is equal. Comparing the Northern European and East Asian samples, they find that Q is statistically indistinguishable from the expectation of 0.75 (ref. 9). However, comparing either non-African population with the West African samples, Q drops significantly below 0.75, which suggests faster genetic drift for the X chromosome in populations outside of Africa. Keinan et al.[9] also compared large amounts of sequence data found in public databases for five individuals of Northern European ancestry, four of East Asian ancestry and five of West African ancestry. They find ratios of pairwise sequence diversity close to 0.75 in the West African population and below 0.75 outside of Africa (**Fig. 1**), which supports their SNP analysis. They demonstrate with simulations that demographic models with a smaller effective number of mating females in non-African populations are necessary to explain their results. Some sex-biased forces that could produce this pattern include recurrent long-range male migration from African sources or a shorter generation time for females.

But the story may not be so clear. In a study with a similar goal of comparing X-chromosomal and autosomal genetic variation, Michael Hammer and colleagues[10] analyzed over 210 kilobases of DNA sequence data from across 20 independent regions on the X chromosome and 20 independent regions on the autosomes, sequenced in each of 90 individuals from six geographically diverse populations (**Fig. 1a**)[10]. They estimate the ratio of effective population size on the X chromosome (N_X) and autosomes (N_A) from two summary statistics of the data—the observed divergence between human and orangutan (used to account for variation in mutation rate among chromosomes) and the number of segregating sites in the sample (that is, SNPs in the data). They observe N_X/N_A is above 0.75 for all six surveyed populations, and in three cases it is significantly higher than 0.75 with no systematic out-of-Africa effect. They therefore conclude that the effective population size of females is larger than that of males. They also use simulations to demonstrate that sex-biased processes are needed to explain their observation, and they suggest that larger variance in male reproductive success relative to females (such as would occur under polygyny) may explain the data.
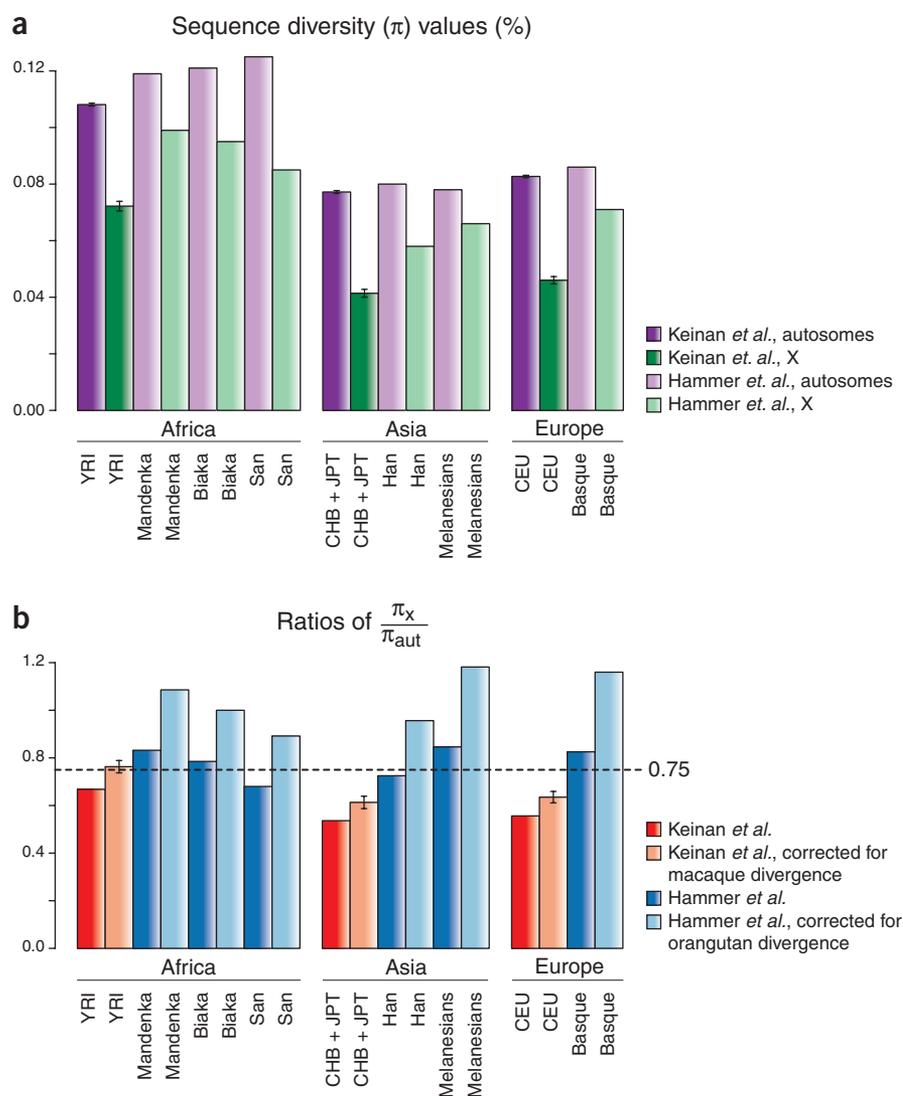
Figure 1 Contrasting patterns of X-chromosomal and autosomal diversity. Data are from Keinan et al.[9] and Hammer et al.[10]. (**a**) Pairwise sequence diversity values for the X chromosome (π_X) and autosomes (π_{aut}). (**b**) Ratios of X-chromosomal to autosomal sequence diversities (π_X/π_{aut}), with and without divergence corrections used by each study. A horizontal line is drawn at 0.75, the expected value for the ratio. YRI, Yoruban (Ibadan, Nigeria); CHB, Han Chinese (Beijing, China); JPT, Japanese (Tokyo, Japan); CEU, CEPH (Utah residents with ancestry from Northern and Western Europe)[11,12].

Finding common ground

In order to reconcile these findings, we compared a common summary statistic, π (pairwise nucleotide diversity), of the data reported by the two groups (**Fig. 1**). While the estimated autosomal nucleotide diversity (π_{aut}) is very similar for both studies ($\approx0.12\%$ per site or 1 SNP per 900 base pairs in African populations, and $\approx0.08\%$ or 1 SNP per 1,250 base pairs in European and East Asian populations), the estimated X-chromosomal nucleotide diversity (π_X) differs substantially (**Fig. 1a**). Keinan et al.[9] report approximately 0.72, 0.46 and 0.41 SNPs per kilobase of X chromosome in West African, North European and East Asian populations, respectively, whereas Hammer et al.[10] find about

40–50% more nucleotide variation in each of their samples for the closest comparable populations (0.99, 0.71 and 0.58 for Mandenka, Han Chinese and Basque, respectively). Both studies effectively use π_X/π_{aut} to estimate N_e for males and females, so the higher overall levels of X-chromosomal diversity in Hammer et al.[10] explain part of the discrepancy. It appears that the rest of the discrepancy is explained by different normalizations for background mutation rate differences between the X chromosome and autosomes (Hammer et al.[10] used human-orangutan divergence and Keinan et al.[9] used human-macaque divergence) (**Fig. 1b**).

While we are able to identify that the papers differ in their estimates of X-chromosomal

genetic diversity and X-to-autosome mutational bias, how and why these differences arise remain open questions. Part of the answer may lie in the advantages and limitations of each study design. Hammer et al.[10] studied population-level processes with direct resequencing among a relatively large number of individuals (which we advocate), but they investigated a limited number of genomic regions, selected to be far from genes and with high recombination rates to minimize possible confounding effects of natural selection. Keinan et al.[9] used two different sources of genome-wide data, starting with SNP data, which is inherently more difficult to interpret due to unknown ascertainment biases in the data, and we commend their attempt to control for many such biases. They demonstrate further support for the observed signature of faster genetic drift on the X chromosome by comparing nucleotide diversity

among pairs of chromosomes from each population. This approach, however, is less powerful than comparing the full spectrum SNP frequencies one observes in deep resequence data. Encouragingly, the genetic diversity estimates reported by Keinan et al.[9] are nearly identical to those recently reported by Bentley et al.[13] for a pair of fully sequenced X chromosomes of a Northern European female (0.47%).

In order to address continuing questions on the nature of sex-biased processes, full genome sequencing of large numbers of individuals sampled from diverse populations will be needed. The upcoming 1,000 Genomes Project (http://www.1000genomes.org/), for example, will provide orders of magnitude more data for these types of analyses. We share the enthusiasm of the population genetics community that this will bring the potential for resolving continuing questions regarding how human history and

cultural practices have shaped global patterns of genomic diversity.

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Protein demethylation required for DNA methylation

Hans-Rudolf Hotz & Antoine H F M Peters

DNA methylation is an epigenetic mark directing stable, heritable gene silencing through development. A new study uncovers the importance of demethylation of the DNA methyltransferase-1 for maintenance of DNA methylation.

DNA methylation and histone modifications have essential roles in the transcriptional regulation of gene expression. Lysine-specific demethylase-1 (Lsd1), also called KDM1, is an enzyme with specificity toward the di- and monomethylation states of lysine 4 and lysine 9 of histone H3 (H3K4 and H3K9), and of lysine 370 of p53 (ref. 1). Lsd1 serves transcriptional co-activator and co-repressor functions during development[2]. On page 125 of this issue, En Li, Taiping Chen and colleagues[3] identify DNA methyltransferase-1 (Dnmt1) as a novel substrate for Lsd1 and show that demethylation of Dnmt1 is required for the maintenance of global DNA methylation.

A surprise
Previously, Lsd1 homozygous knockout mice were shown to have early embryonic lethality[4]. To better understand the role for Lsd1 in development, Wang et al.[3] generated a conditional Lsd1 knockout (Aof2-deficient) mouse

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model. Aof2-deficient embryonic stem cells (ESCs) displayed defects in proliferation and differentiation and increased levels of apoptosis. H3K4 and H3K9 methylation were largely unchanged, thereby underscoring gene-specific regulatory functions for Lsd1 in development. Surprisingly, the authors observed an almost twofold reduction of global levels of DNA methylation in Aof2-deficient cells. Importantly, upon continuous passage, mutant ESCs showed progressive loss of DNA methylation at all unique and repetitive sequences tested. These results argue for a role of Lsd1 in the maintenance rather than establishment of DNA methylation. Accordingly, Wang et al.[3] observed a reduction in the protein level of Dnmt1, the DNA methyltransferase that methylates newly synthesized DNA after replication. Moreover, metabolic labeling experiments indicated a reduced half-life of Dnmt1 in the absence of Lsd1. As transcript levels and in vitro methyltransferase activity of Dnmt1 appeared unchanged, these data suggest that Lsd1 controls Dnmt1 at the post-translational level. In support of this hypothesis, the authors first demonstrated an interaction between the two proteins by co-immunoprecipitation experiments.

Second, by distinguishing between protein methylation and protein synthesis in metabolic labeling studies, they demonstrated that Dnmt1 is methylated at a considerably higher level in Aof2-deficient cells compared with wild-type cells. These data support a model in which Dnmt1 is subjected to Lsd1-mediated demethylation in vivo (Fig. 1a). It leaves open, however, which enzyme would methylate Dnmt1 in the first place.

Recently, several known histone methyltransferases were shown to also modify non-histone targets[5]. Sequence analysis suggested that Dnmt1 could be a substrate of SET7/9, a methyltransferase with broad target specificity[5]. Structural and enzymatic studies demonstrated that SET7/9 recognizes a conserved K/R-S/T/A motif preceding the lysine substrate and has a tendency to bind aspartates and asparagines following the lysine target[6]. This consensus motif is highly conserved among vertebrates at two positions within Dnmt1 proteins (1094-KTK-1096 and 683-RSK-685 in mouse) (Fig. 1b).

Wang et al.[3] now show that recombinant human SET7/9 is able to methylate the K1096 residue, but not K685. Methylation was largely abrogated in a K1096R substitution mutant, which indicates that K1096