processes in the oceans. We posit that future studies will elucidate the relative importance of biotic (viral infection as in this study) and abiotic [phosphorus limitation (17)] stress conditions, in shaping community structure of marine microbes, through the detection of different classes of stress-specific lipids.

Although the origin of PCD in unicellular organisms is still unclear, its functional conservation among phylogenetically diverse phytoplankton lineages suggests key evolutionary and ecological drivers in aquatic environments (29). The retention and expression of a nearly complete, virus-based drivers in aquatic environments (29). The retention and expression of a nearly complete, virus-based

References and Notes

The equin gene set is similar to those of other eutherian mammals and has a predicted 20,322 experiments probing with the two major horse satellite sequences (fig. S2A, table S8, and SOM text), as if it had not had enough time to acquire satellite DNA. We cytogenetically localized the primary constriction (fig. S2B), then precisely mapped, at the sequence level, the centromeric function using chromatim immunoprecipitation (ChIP)-on-chip experiments (fig. S5). In this region, we found only five sequence gaps [none >200 base pairs (bp)], no protein coding sequences, normal levels of noncoding conserved elements, and typical levels of interspersed repetitive sequences, but no satellite tandem repeated sequences (Fig. 1A). We also found no evidence of accumulation of L1 transposons (I0) or KERV-I elements (I1), which were previously hypothesized to influence ENC formation. We propose that the ECA11 centromere was formed very recently during the evolution of the horse lineage, and, in spite of being functional and stable in all horses, has not yet acquired the marks typical of mammalian centromeres.

Fig. 1. Major findings of the genome analysis. (A) Analysis of the primary centromeric constriction of ECA11: 26,000,000 to 30,000,000 bases. ChIP-on-chip analysis with antibodies against centromeric proteins (CENP-A and CENP-C) shows two regions (136 and 99 kb) bound by kinetochore proteins.

CENP-A
CENP-C

Within equine breed
- Across equine breeds
- Within canine breed
- Human

Horse

American quarter horse
Hanoverian
Belgian draft horse
Thoroughbred
Standardbred

Rottweiler
Irish wolfhound
English springer spaniel
Glen of Imaal terrier
Golden retriever

Arabian
Andalusian
Hokkaido
Icelandic horse
Norwegian fjord horse

Akita
Basenji
Pug

Dog

A

Within equine breed
Across equine breeds
Within canine breed
Human

Within equine breed
Across equine breeds
Within canine breed
Human

American quarter horse
Hanoverian
Belgian draft horse
Thoroughbred
Standardbred

Rottweiler
Irish wolfhound
English springer spaniel
Glen of Imaal terrier
Golden retriever

Arabian
Andalusian
Hokkaido
Icelandic horse
Norwegian fjord horse

Akita
Basenji
Pug
protein-coding genes (ENSEMBL build 52.2b), of which 16,617, 17,106, and 17,106 have evidenced orthology to human, mouse, and dog, respectively. The remainder is composed of projected protein-coding genes, novel protein-coding genes, and pseudogenes. One-to-one orthologs with the human account for 15,027 horse gene predictions (SOM). Transcriptome analysis of eight equine samples confirmed the expression of 87% of the 18,039 nonoverlapping genes predicted by ENSEMBL and 88% of the 169,073 predicted exons. Gene family analysis shows paralogous expansion in horses as compared to both human and bovine (SOM) for several interesting families, such as keratin genes related to the condition of pachyonychia (nail bed thickening) in humans (12), perhaps affecting hoof formation; and opsin genes for photoreception, possibly advantageous for visual perception of predators (table S9).

The history of horse domestication, which has important implications for trait mapping strategies, differs in important ways from that of the domestic dog but is perhaps similar to that of the cow. Horses do not appear to have undergone a tight domestication bottleneck, and the presence of many matrilineal lines in domestic horse history has been postulated (13). Screening the horse Y chromosome revealed a limited number of patrilines, consistent with a strong sex bias in the domestication process (14).

We first generated a single-nucleotide polymorphism (SNP) map of more than one million markers at an average density of one SNP per 2 kb by lightly sequencing seven horses from different breeds and by mining the assembly for SNPs (table S10).

We characterized the haplotype structure within and across breeds by genotyping 1,007 SNPs from 10 regions of the genome (SOM) in 12 populations, including 11 breed sets (each with 24 representatives), and 1 set of individual representatives from 24 other breeds and equids. 98% of SNPs were validated, with an average of 69% being polymorphic in alternate breeds (SOM). Like the bovine (15), within-breed linkage disequilibrium (LD) is moderate, dropping to twice the background levels (r2) at 100 to 150 kb (Fig. 1B). The majority of breeds showed similar LD (SOM and fig. S7), and major haplotypes were frequently shared among diverse populations (Fig. 1C). Based on the length of LD in the horse, the number of haplotypes within haplotype blocks, and the polymorphism rate, power calculations suggest that ~100,000 SNPs are sufficient for association mapping within all breeds as well as across breeds (SOM and fig. S8).

Phylogenetic relationships among breeds were inconsistent across resequenced regions (fig. S9), which is most likely a consequence of the close relationships of horse breeds worldwide. We were unable to phylogenetically separate E. przewalskii from the domesticated horses, despite its different karyotype (2N = 66 versus 2N = 64 for the domesticated horse), which is in agreement with recent findings (16), whereas the donkey (E. africanus) is clearly a distinct taxon (fig. S9, table S14, and SOM text). This suggests that either intermixing of E. przewalskii and E. caballus occurred after subspecies separation or that E. przewalskii is recently derived from E. caballus.

We demonstrated the utility of the equine genome sequence and a SNP map by applying these resources to mutation detection for the Leopard Complex (LP) spotting locus (SOM). LP (Appaloosa spotting) is defined by patterns of white occurring with or without pigmented spots (fig. S10). Homozygosity confers a phenotype associated with congenital stationary night blindness in the Appaloosa breed (17). Fine mapping of a 2-Mb region followed by regional sequence capture and sequencing (300 kb) found no indications of associated copy number variants or insertions or deletions but found 42 associated SNPs. Of these, 21 reside within an associated haplotype near a candidate gene melastatin 1 (TRPM1), which is expressed in the eye and melanocytes (18). Two conserved SNPs may be good candidates for the causal mutation.

Our analysis of the first high-quality draft sequence of a horse (E. caballus) distinguishes E. caballus from earlier eutherian genomes by its large synteny with humans and the identification of a centromere repositioning event that may provide an effective model to study epigenetic factors responsible for centromere function. Our results demonstrate that horse population history has led to across-breed haplotype sharing, increasing the feasibility of across-breed mapping. Mapping projects in the horse are likely to accelerate in the coming years and will identify mutations in genes related to morphology, immunology, and metabolism, which may benefit human health.

References and Notes
2. V. A. Trifanov et al., Chromosome Res. 16, 89 (2008).
11. D. M. Carone et al., Chromosoma 118, 113 (2009).
19. We thank the Kentucky Horse Park and L. Chemnick for samples, L. Gaffney for graphics, and M. Daly for useful discussions. Supported by the National Human Genome Research Institute, the Dorothy Russell Havemeyer Foundation, the Volkswagen Foundation, the Morris Animal Foundation, the Centre di Eccellenza di Genomica in Campo Biomedico e Agrario, and the Progetti di Ricerca Scientifica di Rilevante Interesse Nazionale (PRIN-2006). K.L.T. is the recipient of a European Young Investigator award funded by the European Science Foundation. Sequences have GenBank accession numbers AWJW00000001 to AWJW000010. 1,163,466 discovery SNPs have accession numbers rs6841013 to rs69617090 in dbSNP 130.

Supporting Online Material
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MafB/c-Maf Deficiency Enables Self-Renewal of Differentiated Functional Macrophages

Athar Aziz,† Erinn Soucie,† Sandrine Sarrazin,‡ Michael H. Sieweke‡

In metazoan organisms, terminal differentiation is generally tightly linked to cell cycle exit, whereas the undifferentiated state of pluripotent stem cells is associated with unlimited self-renewal. Here, we report that combined deficiency for the transcription factors MafB and c-Maf enables expanded survival of mature monocyes and macrophages in culture without loss of differentiated phenotype and function. Upon transplantation, the expanded cells are nontumorigenic and contribute to functional macrophage populations in vivo. Small hairpin RNA inactivation shows that continuous proliferation of MafB/c-Maf deficient macrophages requires concomitant up-regulation of two pluripotent stem cell–inducing factors, KLF4 and c-Myc. Our results indicate that MafB/c-MafB deficiency renders self-renewal compatible with terminal differentiation. It thus appears possible to amplify functional differentiated cells without malignant transformation or stem cell intermediates.

The nonproliferative state of terminally differentiated cells is assured by robust, often redundant mechanisms (1, 2), and in rare exceptions where fully mature cells can re-enter the cycle, proliferation remains transient and/or involves de-differentiation (3). It remains unknown what renders differentiated cells refractory to the same mitogen signals that stimulate the proliferation of their direct precursors. For example, the proliferative response of myelomonocytic

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