ice shelves (*34*), and glaciation in Patagonia is reported to have taken place around 6 to 7 Ma (*35*).

Taken together, there is considerable evidence that significant global climate variability during the Late Miocene led to a growth of glaciers. Glaciers would readily form in southeast Greenland because of the combination of high precipitation and high topography in the area, and North Atlantic glaciation likely nucleated in this region rather than further north.

Our new data add considerable constraints to the long-term modeling of North Atlantic glaciation. Consistent with data from Iceland (*36*), they prove that the Middle Miocene was fairly mild, that cooling most likely started shortly after 10 Ma within the early Late Miocene, and that full glacial conditions in southeast Greenland were established around 7 Ma in the middle Late Miocene, contemporaneous with Southern Hemisphere glacial expansion. The Late Miocene and Pliocene climate was variable, with perhaps completely ice-free periods. North Atlantic glaciation may have nucleated in—and for the first million years, been largely restricted to—Greenland. The east-west gradient in the abundance of glacial material off southeast Greenland makes it unlikely that this part of Greenland has provided significant contributions to the northern North Atlantic IRD found elsewhere. Therefore, IRD from off mid-Norway and Svalbard dated to between 5.7 and 4.5 Ma (*8*, *23*) suggests that other North Atlantic ice sheets started to form no later than that time and that they were fully established at about 2.5 Ma.

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Isotopic Evidence for Neogene Hominid Paleoenvironments in the Kenya Rift Valley

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Bipedality, the definitive characteristic of the earliest hominids, has been regarded as an adaptive response to a transition from forested to more-open habitats in East Africa sometime between 12 million and 5 million years ago. Analyses of the stable carbon isotopic composition ($\delta^{13}C$) of paleosol carbonate and organic matter from the Tugen Hills succession in Kenya indicate that a heterogeneous environment with a mix of C3 and C4 plants has persisted for the last 15.5 million years. Open grasslands at no time dominated this portion of the rift valley. The observed $\delta^{13}C$ values offer no evidence for a shift from more-closed C3 environments to C4 grassland habitats. If hominids evolved in East Africa during the Late Miocene, they did so in an ecologically diverse setting.

Explanations for the origin of the Hominidae have invoked many different selective pressures, including bioenergetic (*1*), migratory (*2*), morphological (*3*), and thermoregulatory (*4*) considerations associated with foraging and feeding behavior, as well as aspects of life history patterns (*5*) and social systems (*6*). Implicit in most of these explanations is that as Middle to Late Miocene [15 million to 5 million years ago (Ma)] rain forests in Africa became restricted in distribution, drier and more seasonal woodland and grassland communities became widespread, and overall habitat diversity increased. It has been assumed that the divergence of hominids from the other African apes was associated with terrestrial adaptations to exploit resources in open habitats or in bridging forested patches. Origin of the genus *Homo* has also been

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linked to vegetation shifts resulting from worldwide climatic cooling about 2.4 Ma (*7*). However, in situ paleoenvironmental data from East Africa are rare, particularly for the Late Miocene.

Indirect support for a shift in ecosystems related to increasing low-latitude aridity and seasonality has been inferred from evidence of global climatic change recorded in deep-sea cores (*8–10*). Use of the marine record assumes that local and regional terrestrial environments have consistently tracked global climatic changes and have not been significantly affected by local disruption of East African habitats induced by regional crustal doming, volcanism, and graben formation along the zone of rifting over the last 17 million years. In addition to creating topographically and vegetationally diverse landscapes, orographic changes likely had significant effects on regional atmospheric circulation patterns.

The Tugen Hills succession, exposed west of Lake Baringo in the Kenya Rift Valley (Fig. 1A), is the only well-calibrated African sequence spanning the Late Miocene and Pliocene (*11*). This sequence has yielded an extensive faunal record that includes the greatest concentration of hominoids in the 14- to 4-Ma range (*12*) as well as a number of hominid specimens from about 4.5 to 0.2 Ma (*13–16*) (Fig. 1B). Paleoecological data from this succession, linked directly to fossil hominoids, are thus highly relevant to the determination of whether environmental change was a pivotal driving force in hominid evolution.

We determined the stable carbon isotopic composition of soil carbonates and soil organic matter to infer the proportion of vegetation that used the C3 and C4 pathways of carbon fixation (*17–19*). This approach relies on the differential isotopic discrimination during assimilation of atmospheric carbon into C3 and C4 plant tissue (*20, 21*). At soil respiration rates typical for temperate and subtropical ecosystems, the $\delta^{13}C$ of the soil atmosphere (*22*) is overwhelmingly a function of respired CO_2 derived from the microbial oxidation of soil organic matter and root respiration. Because the gas phase controls the isotopic composition of precipitating pedogenic carbonate, the $\delta^{13}C$ of soil carbonate reflects

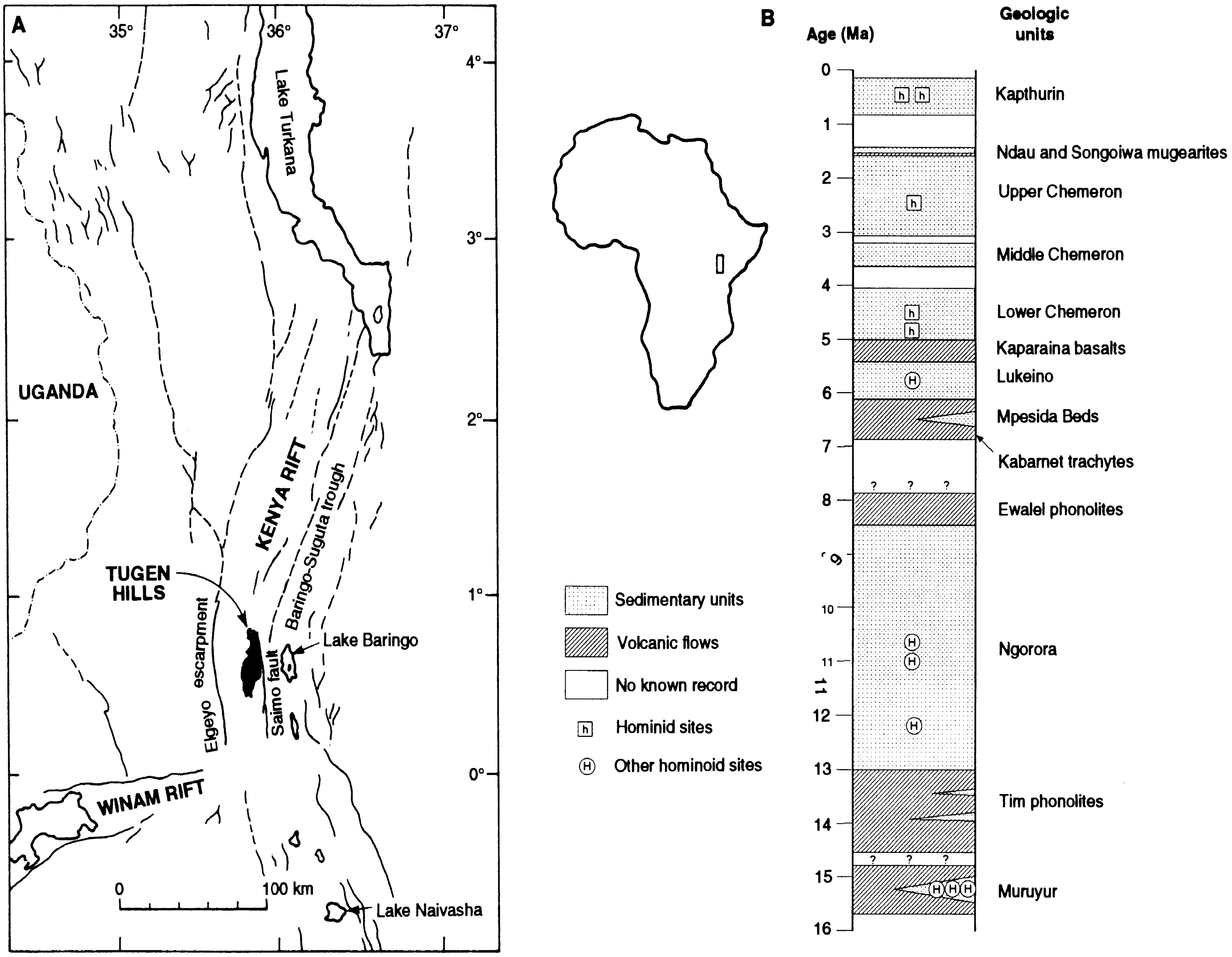

Fig. 1. (**A**) Outline structural map of northwest Kenya showing the location of the Tugen Hills as a complex fault block between the Elgeyo escarpment and the axial Baringo-Suguta trough within the East African Rift Valley. (**B**) Schematic stratigraphic section of the Tugen Hills succession showing sedimentary and volcanic flows units. Geochronology is based on biostratigraphy, radiometric K/Ar and $^{40}Ar/^{39}Ar$ dating, and paleomagnetic studies (*11, 46*). Paleosols exposed along the eastern foothills of the Tugen Hills were formed by modification of volcaniclastic alluvial fan, fluvial, and lacustrine facies and comprise a minor component (<1%) of the sedimentary sequence. Criteria for recognition of paleosols included destruction of primary sedimentary features, bioturbation, root mottling, slickensides, root casts and traces, clay skins, pedogenic nodules, truncation of upper horizons by erosional surfaces, and gradational contacts with underlying units. Organic-rich A horizons were rare. Paleosols are unevenly distributed laterally and vertically in the succession, reflecting complex paleotopography and extremely localized depositional regimes within the structurally complex inner rift.

the relative proportion of surface vegetation using each photosynthetic pathway. Simple models have been used to describe the influence of temperature and other factors on the δ^{13}C of the final carbonate phase (*17*). This approach has been used to reconstruct vegetation in a number of contexts with regional and global significance (*23–26*).

In modern ecosystems, the C3 photosynthetic pathway is used by most trees, shrubs, and herbs as well as grasses preferring wet, cool growing seasons, whereas C4 physiology is linked almost exclusively to grasses and sedges in warm, arid, open habitats. The CO_2 concentrating mechanism of C4 plants is regarded as an adaptation to depressed atmospheric CO_2 levels relative to O_2 or to water-stressed environments (*27, 28*) and is considered to have evolved independently from C3 plants as many as 26 times (*29, 30*). The specific link between C4 vegetation and open habitats provides a key here for documenting the development of either grassland or open woodland habitats (*31*).

To establish a modern analog for interpretation of isotopic data from an inner rift ecosystem, we analyzed the δ^{13}C of modern soil carbonate and associated organic matter from 14 sites along a roughly 20-km transect running north-south through the eastern foothills of the Tugen Hills (elevations ranging from 1200 to 1600 m). The δ^{13}C values of soil carbonates range from −8.8 to +0.4 per mil (Fig. 2), reflecting mixed C3 and C4 vegetation unevenly distributed over the landscape. This variation is also apparent in the carbon isotopic composition of coexisting organic matter (Fig. 3). Both the carbonate and organic matter data are consistent with undisturbed ecosystems in the Baringo Basin characterized by *Acacia reficiens–A. mellifera* bushland and minor riverine woodland with a significant substratum of perennial and annual C4 grasses (*32*). Soils typically form over hundreds or thousands of years, and carbon isotopic analysis of pedogenic carbonate and organic matter reflects proportions of C3 and C4 vegetation averaged over an interval spanning many generations of plants. The wide range of carbon isotopic signatures, despite this averaging effect, indicate that plant heterogeneity in the Tugen Hills region is controlled by factors such as local variation in drainage, bedrock, topography, and rainfall, which persist over longer time scales than soil horizons. Although the modern data cannot provide an exact analog for the reconstruction of past vegetation in the Tugen Hills (range of δ^{13}C variation in modern soil components is shifted toward a greater C4 component relative to paleosols), these data provide an illustration of the expected range of variation across a vegetationally heterogeneous landscape and emphasize the need for extensive lateral isotopic data in geomorphologically complex regions such as continental rift zones.

Exposures of the Tugen Hills succession are locally extensive (>800 km^2), permitting lateral sampling (1 to 8 km) throughout the sequence. The δ^{13}C values of pedogenic carbonate sampled from 95 paleosol sites (Fig. 2) indicate extensive lateral variation at all stratigraphic levels. The observed variance for δ^{13}C values suggests a persistent mosaic of both C3 and C4 plants (averaging ~70 to 80% C3) most closely resembling the estimated range of modern grassy woodlands (*33*). Analyses of variance, both parametric and nonparametric, indicate that there is no statistically significant difference in δ^{13}C variation through the succession except for the modern carbonates, which are enriched in ^{13}C (greater C4 component) relative to the isotopic composition of Chemeron Formation nodules. The isotopic data do not indicate any abrupt vegetational shifts correlating to global climatic shifts, such as the Messinian salinity crises at 5.5 to 4.8 Ma (*34*) or putative cooling at about 2.4 Ma (*35*). Tugen Hills δ^{13}C values also do not reflect a dramatic ecological shift from C3- to C4-dominated floodplain vegetation in northern Pakistan beginning about 7.4 Ma, determined on the basis of paleosol carbonates (*23*), which has been linked to a global decrease in atmospheric CO_2 (*36*).

Results of previous isotopic analyses of paleosol carbonates from hominoid localities in East Africa (*33*) imply that the proportion of C4 biomass has increased gradually over the last 10 million years. Unlike the Tugen Hills record, these data

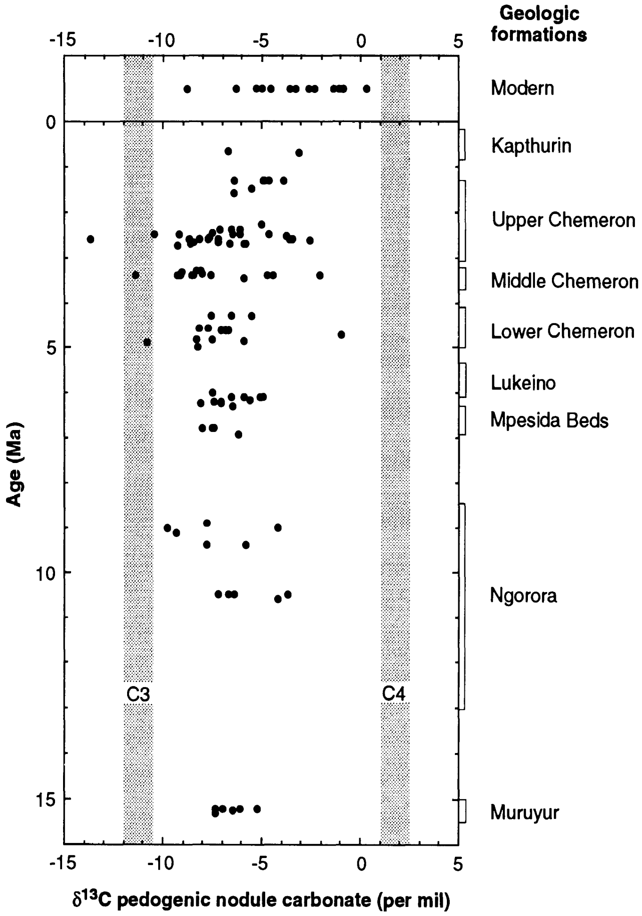

Fig. 2. Stable carbon isotopic composition of paleosol carbonates from the Tugen Hills succession. As a result of ^{13}C enrichment from fractionation during gaseous diffusion and carbonate precipitation, pedogenic carbonates forming at 35° and 15°C are enriched by about 14 and 15.5 per mil, respectively, relative to biologically respired CO_2. Assuming an average δ^{13}C value of −26 per mil for C3 vegetation and −13 per mil for C4 plants, pedogenic nodules forming in C3 and C4 dominated environments have average δ^{13}C values of −12 to −10.5 per mil and +1 to +2.5 per mil, respectively (shaded intervals). Intermediate values indicate a mix of C3 and C4 vegetation. Precise quantitative estimates of the proportion of C4 vegetation inferred from pedogenic carbonate δ^{13}C values are usually not attainable because (i) the range of values for C4 (−15 to −10 per mil) and C3 vegetation (−35 to −23 per mil), (ii) temperature-sensitive fractionation effects during carbonate precipitation, and (iii) assumptions regarding a +1.5 per mil correction for preindustrial atmospheric δ^{13}C values. However, even in the case where C3 vegetation is isotopically heavy, with a δ^{13}C of −23 per mil, and the δ^{13}C of preindustrial atmospheric CO_2 is −6.5 per mil (*47*), the δ^{13}C values of paleosol carbonates forming at 15° to 35°C fall outside the potential pure C3 range from horizons throughout the sequence, indicating a C4 component as early as 15.3 Ma. Crassulacian acid metabolism (CAM) plants, with δ^{13}C values spanning the range of C3 and C4 vegetation, are adapted to extreme xeric conditions and we assume were unlikely to have comprised a significant component of the plant biomass in the Tugen Hills during the Neogene. Analyses of three standards [one internal standard, NBS (National Bureau of Standards) 19, and NBS 20] in conjunction with carbonate samples yielded δ^{13}C SD of ±0.15 (n = 66), ±0.09 (n = 12), and ±0.21 per mil (n = 15), respectively. The SD of nine samples from a single carbonate nodule was ±0.31 per mil, and replicate analyses of 29 nodules resulted in a SD of ±0.13 per mil.

are a compilation of $\delta^{13}C$ values from sites separated by up to 750 km. At a number of these East African sites, the proportion of C4 biomass differs substantially from what we observed at roughly contemporaneous horizons in the Tugen Hills succession, suggesting regional vegetation heterogeneity and offering additional support for the notion that early hominids exploited a range of habitats.

Carbon isotopic analysis of bulk paleosol organic material (disseminated and nodular) (Fig. 3) corroborates the lateral variation shown by $\delta^{13}C$ values of pedogenic carbonate, indicating that habitats in the Tugen Hills were heterogeneous, a mix of C3 and C4 vegetation. The $\delta^{13}C$ organic carbon values for samples older than 8.5 Ma reflect a slightly greater C3 component than do associated pedogenic carbonates. These data, if representative of the original vegetation, suggest an increase in the proportion of C4 vegetation between 8.5 and 6.8 Ma. Discrepancy between the $\delta^{13}C$ values of paleosol carbonate and coexisting organic matter can be attributed to either diagenetic modification of soil components or deviation from model conditions during carbonate precipitation (*37*). Careful sampling strategy (*38*) has minimized effects of the latter. Research has shown carbonate nodules to be impervious to significant diagenesis (*39*) relative to organic matter, which can be altered (*40*).

Isotopic analysis of pedogenic carbonate throughout the Tugen Hills succession indicates that ecosystems contained a mix of C3 and C4 vegetation, but the specifics of these paleoenvironments remain unknown. Potential habitats, consistent with our current knowledge of the relation between the $\delta^{13}C$ of soil components and vegetation, range from woodland to semidesert bushland. In addition, some habitats are not represented in the paleosol carbonate record. For example, an extensive autochthonous paleoflora from a 12.6-Ma horizon in the Tugen Hills includes 57 taxa with rain forest affinities (*41*). This flora suggests high rainfall conditions that do not favor the formation or preservation of pedogenic carbonate, and the local sediments are devoid of carbonate bearing paleosols. Forests may have formed an integral part of past ecosystems in the area, perhaps dominating escarpment ridges as they do today. Interpretations of paleohabitats based on isotopic data derived from paleosols rely on the uniformitarian assumption that ecological and climatic affinities as well as isotopic signatures of C3 and C4 vegetation have remained consistent through the Neogene. However, under different atmospheric conditions and before the evolution of C4 metabolism, C3 plants or C3-C4 intermediates may have occupied the modern C4 niche and formed extensive temperate and tropical grasslands.

Isotopic paleosol data from the Tugen Hills, incorporating lateral sampling over a long time span in a single widespread locality, document a complex vegetational mosaic of C3 and C4 plants over the past 15.3 Ma in the Baringo Basin with no dominant vegetational trend. This reconstruction of rift valley vegetation does not rely on a compilation of data from spatiotemporally limited sites, which is problematic in vegetationally heterogeneous regions such as the East African Rift system. The presence of C4 vegetation at 15.3 Ma, representing the oldest evidence for C4 flora, is supported by stable carbon isotopic analysis of fossil herbivore tooth apatite from the Tugen Hills (*42*). Although extremely poor, the paleobotanical record of East Africa (*43*, *44*) as well faunal and sedimentological data (*45*) indicate ecological diversity during the Miocene with arid-adapted communities established as early as 23 Ma. While the course of human evolution was surely affected by environmental change, our data suggest that interpretations of the origin of hominids in East Africa during the Late Miocene should be considered within the context of a heterogeneous mosaic of environments rather than an abrupt replacement of rain forests by grassland and woodland biomes.

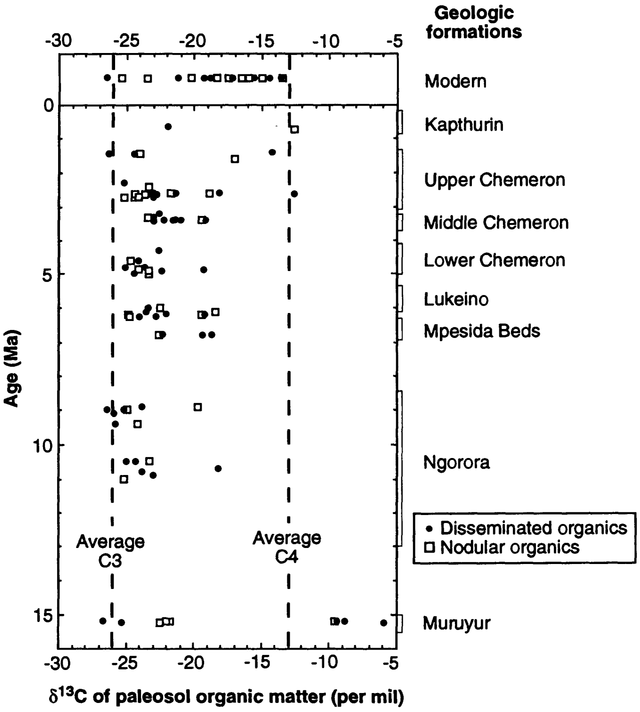

Fig. 3. The $\delta^{13}C$ of paleosol organic matter, (●) disseminated and (□) nodular, from the Tugen Hills succession. The C3 and C4 ranges are based on analyses of the carbon isotopic composition of modern C3 and C4 vegetation collected in the East African Rift Valley (*25*). Bulk nodular organics, isolated by dissolution of pedogenic carbonate nodules in HCl, may have greater potential than disseminated carbon residue for accurately retaining the $\delta^{13}C$ of the original humus as it is relatively sequestered from diagenetic processes. Difference in the $\delta^{13}C$ of organic matter and pedogenic carbonate ($\Delta\delta$) has been used as a test of the state of preservation of the original ecological signal in paleosol components (*37*). Theoretical models predict a $\Delta\delta$ of 14 and 15.5 per mil at 35° and 15°C, respectively. Although means for the $\Delta\delta$ for disseminated and nodular organics are within the theoretical range (15.3 and 15.4 per mil, respectively), the standard deviation is substantial (5.0 and 4.1 per mil, respectively). Four organic residues from the Muruyur Formation (15.3 to 15.2 Ma) yielded $\delta^{13}C$ values greater than that of any modern whole plant tissue (< -10 per mil), suggesting diagenetic ^{13}C enrichment. Analysis of an internal standard ($n = 14$) with organic samples had a $\delta^{13}C$ standard deviation of ±0.07 per mil.

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$$\delta^{13}C = \left[\frac{(^{13}C/^{12}C)_{\text{sample}}}{(^{13}C/^{12}C)_{\text{std}}} - 1\right] \times 1000$$

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38. A key aspect of this study involves differentiating pedogenic carbonate from detrital or groundwater origin carbonates. The theoretical model linking vegetation to pedogenic carbonate (*17*) assumes carbonate formation in the unsaturated zone where the isotope species distribution is determined by gaseous diffusion. The original parent material of paleosols in the Tugen Hills was largely noncalcareous, and features such as leached zones were not consistently evident. The lack of parent carbonate in the sequence, however, suggests that potential contamination by nonpedogenic carbonate is minimal. Carbonate nodules were sampled at depths >40 cm to minimize potential mixing of isotopically heavy atmospheric CO_2 with biologically respired CO_2 in soil systems.
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40. Models relating the $\delta^{13}C$ values of bulk organic matter in paleosols to those in paleovegetation generally assume that organic residues in sediments retain the isotopic signature of original plant material. However, preferential preservation of lignoproteins and lipids, which can have $\delta^{13}C$ values 2 to 6 per mil less than the whole plant [R. Benner, M. L. Fogel, E. K. Sprague, R. E. Hodson, *Nature* **329**, 708 (1987)], as well as decarboxylation reactions during diagenesis [P. Deines, in *The Terrestrial Environment*, vol. 1 of *Handbook of Environmental Isotopic Geochemistry*, P. Fritz and J. C. Fontes, Eds. (Elsevier, New York, 1980), pp. 329–406] can lead to ^{13}C depletion in residual matter. Alternatively, preferential loss of ^{12}C during microbial oxidation can result in residual carbon enrichment by a few per mil relative to plant biomass [K. J. Nadelhoffer and B. Fry, *Soil Sci. Soc. Am. J.* **52**, 1633 (1988)]. Under these conditions, the $\delta^{13}C$ difference between soil carbonate and associated organic matter would deviate from model predictions.
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48. We thank K. Cheboi, B. Kamoya, P. Kiptalam, and T. Cerling for assistance in the Tugen Hills, and the government of the Republic of Kenya for research permission. Thanks also to D Pilbeam, M. Morgan, and B. Brown for comments. This work was supported by grants from the National Science Foundation (NSF), the LSB Leakey Foundation, Boise Fund, the Geological Society of America, Bill Bishop Memorial Fund, Harvard University Anthropology Department, and Sigma Xi. Additional support was provided by the Baringo Paleontological Research Project funded by NSF (A.H.) and by the U.S. Department of Energy through the National Institute of Global Environmental Change (B.D.M.)

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Retention and 5′ Cap Trimethylation of U3 snRNA in the Nucleus

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It is shown here that maturation of the m^7G-capped precursors of U3 small nuclear RNA (snRNA) occurs by a previously unknown pathway. In contrast to the 5′ m^7G-capped precursors of other snRNAs, this RNA is not exported to the cytoplasm but is retained in the nuclei of *Xenopus laevis* oocytes, where it undergoes trimethylation of its 5′ cap. The m^7G caps of most snRNA precursors are trimethylated only after transport of the RNAs to the cytoplasm. The nuclear retention and maturation of this nucleolar RNA raises the possibility that other m^7G-capped RNAs are also retained and modified in the nucleus.

The m^7G caps at the 5′ ends of RNAs made by RNA polymerase II (RNA Pol II) facilitate the efficient export of many of these transcripts from the nucleus to the cytoplasm (*1, 2*). Precursors of the spliceosomal U1, U2, U4, and U5 small nuclear RNAs (pre-snRNAs), which are made by RNA Pol II, are convenient substrates for the study of nucleocytoplasmic RNA trafficking, because maturation of these RNAs occurs only after they have been exported to the cytoplasm. Trimethylation of their m^7G caps to $m^{2,2,7}G$ caps and transport of the RNAs back to the nucleus require the binding of Sm proteins to a sequence element common to these RNAs (*3, 4*). Curiously, U3 RNA, a nucleolar snRNA that functions in ribosomal RNA processing, has a $m^{2,2,7}G$ cap although the RNA lacks an Sm protein binding site (*5*). Here, we show that in spite of its m^7G cap, newly made pre-U3 RNA remains in the nucleus where trimethylation of the cap occurs. Thus, the nucleolar U3 RNA is processed by a pathway distinct from that of nucleoplasmic snRNAs. This raises the possibility that other m^7G-capped precursors of nucleolar snRNAs also are retained and processed in the nucleus; this result also confirms our previous observation (*1*) that m^7G caps are not sufficient to direct RNA Pol II transcripts out of the nucleus.

To determine the nucleocytoplasmic distribution of newly made U3 RNA, we injected a *X. laevis* U3 gene into nuclei of *X. laevis* oocytes and isolated the resulting RNA from manually dissected (6) cytoplasmic and nuclear fractions (Fig. 1A). No U3 RNA was detected in the cytoplasm, although large amounts of this RNA were present in the nucleus. As expected, a precursor of U1 RNA was present in the cytoplasm, whereas the spliceosomal U6 RNA, which is made by RNA Pol III (*4*) and has a γ-mpppG cap (*7*), was not; this

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