R24C is selectively impaired in its interaction with $p16^{INK4a}$ and $p15^{INK4b}$ and suggest that Arg^{24} is directly involved in binding to $p16^{INK4a}$ and $p15^{INK4b}$.

The cell cycle regulatory pathway that involves the retinoblastoma protein (Rb), cyclin D1, $p16^{INK4a}$, and CDK4 has been implicated in tumorigenesis (*21*). In particular, $p16^{INK4a}$ can inhibit cell proliferation and oncogenic transformation of cultured cells (*22*). Inactivation of the gene encoding $p16^{INK4a}$ is common in some tumor cell lines and primary tumors and is responsible for genetic predisposition to melanoma (*23*). Mutation of CDK4 at positions that disrupt its interaction with $p16^{INK4a}$ may constitute a mechanism to subvert this regulatory pathway in tumor cells. It seems plausible that, aside from its antigenicity, the expression of CDK4-R24C contributed to malignant transformation in melanoma SK29(AV). Antigens derived from oncogenic proteins are ideally suited as targets of tumor rejection responses because tumorigenesis is likely to depend on the continued expression of the antigen. Indeed, CTLs against viral oncoproteins have been demonstrated to elicit rejection response and protective immunity to virally induced murine tumors (*24*). It remains to be proven that human tumor-specific antigens like CDK4-R24C can constitute targets for rejection response in vivo. However, it should be noted that patient SK29(AV) has been free of detectable disease since 1978 (*7*).

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28. Total RNA was extracted from SK29-MEL-1 and SK29-EBV-B cells as described [P. Chomczynski and N. Sacchi, *Anal. Biochem.* **162**, 156 (1987)]. RNA (5 μg) was transcribed with the Superscript preamplification kit (Gibco-BRL) with oligo(dT) primers. Single-stranded cDNA served as a template for PCR with CDK4 primers C2 and C3 (Fig. 2A). Primers contained restriction sites for enzymes Eco RI (C2) or Xba I (C3) in their nonspecific nucleotide regions. PCR products were digested with Eco RI and Xba I and were then cloned in the corresponding sites of vector pcDNAI/Amp (Invitrogen) as described (*27*). Clones B29-2/3 (CDK4-wt) and M29-2/1 (CDK4-R24C) were sequenced and used for transfection.
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30. We thank L. J. Old for melanoma cell line SK29-MEL; J. Rosai, P. Livingston, and P. Schirmacher for paraffin sections of metastasic melanoma tissues; P. Cresswell for mutant cell line 0.174×CEM.T2 (T2); T. Espevik for cell line WEHI 13 (W13); J. W. Drijfhout for synthesis of peptides; G. Hannon for helpful suggestions; and H. Tran for technical assistance. Supported by grant C6/SFB311 from the Deutsche Forschungsgemeinschaft (T.W.) and by a fellowship from the Deutsche Forschungsgemeinschaft (J.S.). D.B. is supported by grants from NIH and is an Investigator of the Howard Hughes Medical Institute.

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TECHNICAL COMMENTS

Lunar Phase Influence on Global Temperatures

In a report by Robert C. Balling Jr. and Randall S. Cerveny about the influence of lunar phase on planetary temperature (*1*), a data set of satellite-derived, lower tropospheric global temperature is presented showing a significant 0.02 K modulation between new moon and full moon. The warmest daily global temperatures occur approximately at the time of full moon. There are several possible explanations of this phenomenon. For instance, the increased solar load resulting from the moon's reflection—as well as the increased infrared emission from the moon's surface—give an effect which is of the correct order of magnitude (*1*).

There is another factor contributing to the global temperature modulation. Earth and moon circulate around their common center of gravity, the barycenter. At full moon the sun, Earth, and moon lie approximately on a line and the Earth is nearest to the sun. As the moon weights 1/81 of the Earth and the Earth-moon distance is 384,000 km, the Earth moves in a circle with radius 4700 km around the barycenter. This induces a modulation in the Earth-sun distance equal to 9400 km. Consider a naïve model in which the Earth absorbs all sunlight and re-radiates it as infrared black body radiation, with balance between incoming and outgoing radiation. The intensity of sunlight received varies as the inverse Earth-sun distance squared, and the intensity of black body radiation varies as the temperature to the fourth power. Thus, for small variations

$$dT/T = -dR/(2R)$$

where T is the temperature and R is the Earth-sun distance. For R = 150,000,000 km and a mean temperature of 269 K this leads to 0.008 K temperature modulation.

Inspection of figure 1 of the report (*1*) shows that the data are noisy. There is some

uncertainty in the number 0.02 K itself; the line drawn on this figure indicates a temperature modulation of only 0.013 K. Although this is close to 0.008 K, the naïve model has a temperature modulation that is too large because the assumption has been made that the temperature response is instantaneous. In a more realistic model, the temperature lags somewhat behind the radiation received because the atmosphere has a finite specific heat and, more important, because the atmosphere is heated mainly from the Earth's surface by slow processes like thermals and latent heating in precipitation after evaporation. Also, any response of the oceans would take months.

The phase lag means that the temperature modulation that is due to the fact that the Earth is nearer to the sun at full moon than at new moon is less than 0.008 K. Still, it is possible that this effect, in conjunction with increased radiation received from the full moon, may explain the observed global temperature modulation. It may not be necessary to invoke explanations like feedback responses of global temperature to potentially lunar-related variations in climatic parameters such as precipitation, cloudiness, and thunderstorm activity (*1*).

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About half of the 0.02 K signal noted by Balling and Cerveny can be attributed to the long-known McConnell effect, whereby Earth's motion about the center-of-mass of the Earth-moon system places it about 4700 km closer to (or further from) the sun at the full (or new) moon than it is on average. Using only the inverse square law, the Stefan-Boltzmann law, and well-known properties of the Earth-moon system, the McConnell effect predicts

$$\Delta T/T = \Delta E/4E = -\Delta R/2R$$

where ΔT is the perturbation of the mean temperature T, ΔE is the perturbation radiant energy, and ΔR is the perturbation of the R = 149 Gm Earth-Sol distance ($\Delta R = \pm 4.7$ Mm for motion about the barycenter). With tropospheric T = 269 K, the McConnell effect predicts a full-minus-new moon global warming of 0.0085 K.

The effect clearly presumes a balance between incoming and outgoing radiant energy at monthly periods; yet it precludes neither time lag, attenuation, nor spectral line-broadening corrections resulting from heat conduction into the land, thermocompositional tropospheric mixing, or even a hydro-cryospheric response.

The time lag is the driving period multiplied by a phase factor ($\phi/2\pi$) which, in some cases, need not vary much over a limited range of periods. Common experience of a daily temperature maximum a couple of hours after local noon suggests this factor is about 0.1. This value predicts a lag in the response to annual forcing of about a month, which is not too far from common experience of a seasonal maximum temperature a month or two after summer solstice. It further predicts a time lag for the much smaller amplitude, intermediate period McConnell effect that is no more than a few days. Isolation of the response to the modulation of insolation by motion about the barycenter, including an accurate determination of the time lag, might help constrain detailed models of the coupled terra-hydro-cryo-atmospheric system.

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Response: In addition to the mechanisms presented in our report (*1*), we agree that the barycenter movement effect discussed by Dyre and Voorhies should be considered in any explanation of the impact of lunar phase on daily global temperatures. However, our research on this problem still suggests, as we discussed in our initial paper, that the most probable explanation for the moon's influence on global temperature involves multiple controls and feedback processes. Two calculations demonstrate this point.

First, the satellite-based daily data reveal that the mean global temperature is highest approximately 24 days after the full moon, or about 5 days before the full moon. This indicates the presence of indirect feedback mechanisms existing bewteen insolation and tropospheric temperature. Second, using a new latitudinal daily tropospheric temperature dataset, we have identified strong geographic variations in the timing of temperature maxima in relation to lunar phase. In the mid-latitudes of both hemispheres, the peak lower tropospheric temperature occurs near the time of the new moon, while the equatorial and polar regions have highest temperatures near the time of the full moon. These spatial differences are difficult to explain in the absence of feedback mechanisms.

While the barycenter effect must be considered, we believe, as we stated in our report, that a more complex explanation is necessary to account for the relationship between lunar phase and tropospheric temperatures. Continued research will undoubtedly add to our understanding of lunar-climate interactions.

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