the mutation rate must be very high, as only a small proportion of cells in these cultures (7.5%) have an S-phase DNA content. The high level of microsatellite instability in subclones isolated from high-density cultures and the *HPRT* mutant strains appears to be consistent with the idea that widespread catastrophic DNA synthesis occurs in these cells. Thus, the conditional mutator phenotype may reflect the loss of a checkpoint that prevents cells from entering the S phase when environmental conditions are not optimal, which is not unlike the checkpoint that arrests cells exposed to hypoxia (*23*). With respect to the possibility of error-prone repair in cells maintained at high density, it was recently reported that transient exposure of a mouse tumor cell line to hypoxia modestly increased the mutant frequency of a target gene (*24*). Mismatch repair has thus far been primarily associated with correction of DNA replication errors in growing cells, but may have another function or functions outside the S phase (*25*).

Our observations raise the possibility that mutations in some cells may accumulate in a time-dependent manner in the absence of growth, as proposed by Strauss (*26*). Furthermore, because the majority of cells in a tumor may not be in a microenvironment conducive to the rapid growth that occurs in cell culture, the conditions in high-density cultures described here may more closely resemble conditions in the tumor.

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11. Most of the cell lines used in these experiments were obtained from the American Type Culture Collection. MRC-5 was obtained from the Imperial Cancer Research Fund Cell Production Facility. 2774 was the kind gift of T. Kunkel. All cultures were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Mutation rates were determined by the Luria-Delbrück fluctuation test [S. E. Luria and M. Delbrück, *Genetics* **28**, 491 (1943)]. Inocula containing 100 cells were grown for 2 weeks on 35-mm tissue culture wells. Colonies formed after this time were treated with trypsin and transferred to 100-mm dishes. After 5 days, the replica cultures were plated on 6-thioguanine (5 μg/ml) to determine mutation rate at the *HPRT* locus or on 1 μM ouabain to determine the rate of mutation to resistance to this drug. After 2 weeks, plates were stained and colonies larger than 50 cells were counted. The mutation rate was calculated by the method of the mean [R. L. Capizzi and J. W. Jameson, *Mutat. Res.* **17**, 147 (1973)].
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18. B. Richards, unpublished data. The rate of mutation to *Oua*^{R} was elevated 24- to 35-fold in tumor cells deficient in other mismatch repair components.
19. Analysis of *HPRT* mutations was performed as in (*20*), except that an ABI automated sequencer (Model 377) was used to determine the base alterations. Positions of mutations in the coding sequence [D. Jolly *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 477 (1983)] are presented with the A in the ATG initiation codon being nucleotide 1. Mutations in the introns that alter splicing are numbered with respect to the *HPRT* genomic sequence [A. Edwards *et al.*, *Genomics* **6**, 593 (1990)].
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21. Microsatellite instability was analyzed as in (*20*). DNA purified from cultures grown from single cells of the indicated lines was used to amplify microsatellite loci. Four loci were assayed: *BAT26*, which contains a mononucleotide run; *D10S197* and *D16S521*, which are both dinucleotide repeats; and *D7S1794*, which contains a trinucleotide repeat. The frequency of instability presented in the text represents the fraction of subclones from each of the lines exhibiting novel alleles at the loci. Patterns of alleles at one of the microsatellite loci *(D10S197)* in single cell clones isolated from 2774 are presented in Fig. 1.
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TECHNICAL COMMENTS

Observations of Emission Bands in Comet Hale-Bopp

Heike Rauer *et al.* observed anomalous H_2O^+ and NH_2 emission bands in comet Hale-Bopp (C/1995 01) at large heliocentric distances before perihelion (*1*). The subject bands are from bending vibrational transitions, $(0,\upsilon_2',0)$-$(0,0,0)$, of the $\tilde{A}^2A_1 - \tilde{X}^2B_1$ system of the two isoelectronic molecular species. Except at the shortest heliocentric distances studied, only emissions from even bending vibrational levels were observed, a phenomenon for which existing fluorescence excitation models provide no explanation, according to Rauer *et al.*

An equivalent observation, however, was made in comet Kohoutek by Wehinger *et al.* (*2*), who identified H_2O^+ and attributed the phenomenon to fluorescence excitation of molecules at temperatures below 50 K. The reason for the missing vibrational bands can be found in the electronic structure of H_2O^+ and NH_2 (*3*, *4*) that gives rise to optical transitions involving a lower state bent asymmetric rotor with quantum numbers J'', N'', K_a'', and K_c'' and an upper state linear symmetric rotor with quantum numbers J', N', and K', the latter quantum number being equivalent to K_a'. While there is no vibrational level-dependent constraint on K_a'' values in the ground electronic state, odd K' vibronic sublevels of the linear excited state are restricted to even bending vibrational states, while even K' sublevels are associated with odd bender states.

Given the $\Delta K_a = \pm 1$ selection rule of the H_2O^+ and NH_2 $\tilde{A}^2A_1 - \tilde{X}^2B_1$ transitions, absorption to the unobserved odd

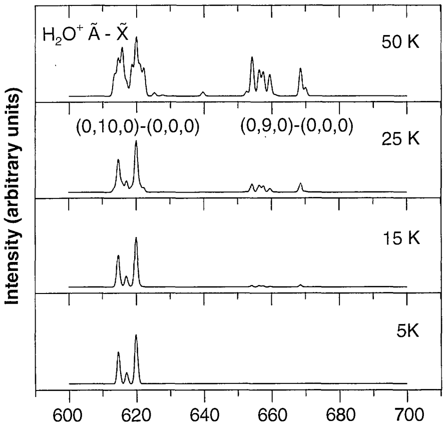

Fig. 1. Fluorescence excitation spectra calculated for the H_2O^+ $\tilde{A}^2A_1 - \tilde{X}^2B_1$ system at four temperatures of the ground-state molecule. Calculation assumes 1-nm resolution and a uniform spectral sensitivity.

bender states can only occur from odd K_a'' levels of the lower state. Population of K_a'' = 1 states requires a minimum rotational excitation of 37 cm^{-1} for H_2O^+ (*4*) [32 cm^{-1} for NH_2 (*3*)], corresponding to a temperature of 53 K. We calculated (Fig. 1) the temperature dependence of H_2O^+ fluorescence excitation spectra for the same spectral range in which H_2O^+ emissions were observed by Rauer *et al.* (*1*). The calculations use term values, frequencies, line strengths given by Lew (*4*), and the methodology described by Dressler *et al.* (*5*, *6*). The H_2O^+ $\tilde{A}-$ state bending vibrational assignment adopted by Rauer *et al.* has recently been revised (*7*, *8*). The (0,9,0)-(0,0,0) band becomes apparent between 15 and ~25 K, suggesting that odd bands should be visible closer to perihelion. This was indeed the case for Kohoutek (*2*) and was also observed by Rauer *et al.* in Hale-Bopp for NH_2 at $r_h < 3$ AU (*1*).

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Response: We thank Dressler for his interesting comment to our observation of the excitation of NH_2 and H_2O^+ in comet Hale-Bopp. His calculation shows that odd vibrational bands should become visible at temperatures larger than 25 K and might therefore be visible closer to perihelion as the temperature in the coma increases. However, we would like to point out in more detail than we could in our short report (*1*) that a straightforward interpretation of the observations is not possible and a more detailed model of the excitation in a cometary coma is required.

The calculation by Dressler assumes thermal populations of the lower rotational levels and determines relative line intensities under fluorescence excited by an unspecified source. In such a case, the input kinetic temperature, T_k, is equivalent to the rotational excitation temperature, T_{exc}, in the computed spectrum. However, in nonthermal equilibrium conditions as they are found throughout most of the cometary coma, T_{exc}, determined by the relative population of the rotational or vibrational levels, is not generally equivalent to T_k. While the absence of the odd bands in a comet at large heliocentric distance is indicative of a low rotational T_{exc} as we pointed out [page 1911, column 3, paragraph 3 in our report (*1*)], it does not allow any conclusion to be drawn as to a thermal excitation at a corresponding T_k.

In comets, a temperature-dependent excitation should play a role only in the innermost, collisionally dominated coma. In comet Hale-Bopp, this collisionally dominated region is larger than that in other comets at the same heliocentric distance range as a result of its higher gas production rate. However, throughout most of the coma and in the ion tail, the populations of all the levels—and therefore the NH_2 and H_2O^+ emissions—are governed by purely radiative processes; they depend on the incoming solar radiation as well as on the molecular characteristics, notably the strength of pure rotational transitions within the ground state (*2*). Existing resonance fluorescence models (*3*) are unable to reproduce the observed visibility of even and odd bands, mainly because they do not account for the rotational structure of the molecule or ion.

A realistic excitation model of NH_2 and H_2O^+ in a cometary coma and ion tail must take into account that thermal rotational equilibrium does not hold in such low density environments. The temporal evolution of the population of rotational levels with increasing nucleocentric distance must be taken into account, in addition to the pumping by solar flux as a result of resonance fluorescence processes and the rotational de-excitation processes mentioned above. The latter are important in the species in hand because they are in the hydride radicals OH, NH, and CH. The spectra modeled by Dressler show the effect of temperature-dependent excitation in thermal equilibrium conditions for NH_2 and H_2O^+. However, only a detailed investigation including all significant excitation processes, considering the spatial distribution of the emissions, and covering a range of heliocentric distances will provide a full explanation of the observations.

A correct treatment of the populations of even and odd levels for NH_2 and H_2O^+ is important. Neglecting to account for the appropriate selection rules leads to production rates that are underestimated by a factor of about 2 near 1 AU from the sun (*4*). For H_2O^+ it was shown (*5*) that the large discrepancy of the ion production rates in comparison to its parent, water, is most likely caused by incorrect *g*-factors for the emission bands observed. Furthermore, the observations of comet Kohoutek (*6*), quoted by Dressler, showed that odd bands of H_2O^+ were weak in a comet still at heliocentric distances of about 1.4 AU. Some other spectra of the same comet and of comet West at the same r_h, however, showed these bands (also those of NH_2) with comparable intensities to the even bands (*7*). A similar remark can be made regarding comet West near 1.6 AU (*8*).

Finally, we would like to thank Dressler for making us aware of the changed assignments of vibrational levels for H_2O^+.

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