The phenomenon of *trans*-acting transcriptional activation occurs in several DNA viruses. Transcription from the adenovirus early transcriptional units is increased by the Ela gene product (*22*), and similar transcriptional enhancement occurs with the immediate early gene of herpes simplex virus type I (*23*). There is also evidence that the SV40 T antigen activates transcription of late region genes of SV40 (*24*). The recent discovery of *trans*-acting transcriptional activation in several retroviral systems has prompted speculation that these viruses, like some DNA viruses, code for their own *trans*-acting enhancer proteins (*5–7*). In support of this theory, spliced RNA transcripts and protein products encoded by additional open reading frames have been detected in some infected cells (*25*). In the case of RSV, a region of the viral genome has been identified which activates transcription in *trans* from a plasmid containing the viral LTR (*9*). Our experiments suggest that cells infected with visna virus also produce a *trans*-acting factor. However, we have not identified a viral gene product responsible for transcriptional activation of the visna LTR, and we cannot exclude the possibility that the *trans*-acting transcriptional activator is a cellular protein that is induced by viral infection.

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19. Cells grown in monolayers were harvested for CAT assay by trypsinzation. The cells were washed twice with cold PBS (*p*H 7.4), then lysed by freeze-thawing three times in 60 μl of 250 m*M* tris-HCl (*p*H 7.8). Cell debris was pelleted by centrifugation, and 30 μl of the cellular supernatant was assayed for CAT activity as described (*14, 15*). The percentage acetylation of ^{14}C-labeled chloramphenicol (New England Nuclear) after 1 or 2 hours was measured by separating the acetylated and unacetylated forms by thin-layer chromatography, then counting spots cut from the plate by liquid scintillation. The results are expressed as the percentage conversion of ^{14}C-labeled chloramphenicol to its acetylated derivatives per hour normalized to the percentage conversion by either pRSVCAT (Table 1) or pSV1CAT (Table 2).
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19 February 1985; accepted 29 May 1985

Carbyne Forms of Carbon: Evidence for Their Existence

The report by Smith and Buseck (*1*) casts doubt on the existence of carbyne forms of carbon. However, the existence of this carbon form was clearly established in a number of ways many years ago. In a laser-Raman study, Nakamizo *et al.* showed that a solid form of carbon containing triple bonds could be prepared (*2*). This, by definition, is a carbyne form of carbon. Russian investigators produced a large body of data on the preparation and properties of carbynes. Most of their work is summarized in a review article by Sladkov (*3*), which gives data on the electronic structure, infrared and Raman spectra, and electrophysical, thermo-physical, and chemical properties of carbynes. It is difficult to understand how so many data can have been obtained by a dozen or more investigators on something that does not exist.

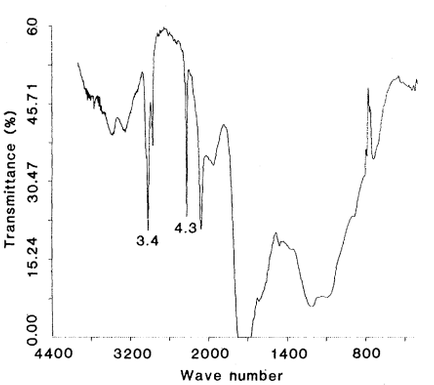

Fig. 1. Infrared spectrum of a carbyne film (*4*).

Recently I have been engaged in a study of carbyne films prepared by quenching carbon gas on a polished metal substrate. Absorption data were obtained on these films from 0.2 to 25 μm. An infrared spectrum of a carbyne film is shown in Fig. 1 (*4*). The spectrum shows features different from those found in the spectra of graphite and diamond. In particular, they show the –C≡C– resonance at ~4.3 μm and a feature that occurs at ~3.4 μm. This is close to the value of 3.1 μm predicted by Webster on theoretical grounds (*5*). In addition, these films gave ion-probe spectra showing carbon molecule ions (no silicate fragments) and electron diffraction patterns that correspond to those associated with carbynes (*6, 7*).

Smith and Buseck did not make use of all the diffraction data available. Had they done this, they would not have confused chaoite with other minerals. Since they used nontronite and quartz extensively in their report, I will use these minerals to illustrate this point. It is rather surprising that Smith and Buseck did not include the strongest reflection for both nontronite and α quartz in their table 1 (*1*). If the strongest reflection for nontronite (d_{001} = 14.7 Å) had been included, it would have been immediately obvious that chaoite (or any other carbyne) could not be confused with

nontronite or any nontronite-quartz mixture because chaoite has no allowed reflection at a *d*-value anywhere near as large as 14.7 Å. Smith and Buseck used only *d*-values in their analysis, but this can lead to incorrect results. Intensities must be considered also. Comparison of the radial distribution of intensities for powder patterns of chaoite, nontronite, and α quartz show very little similarity even though many *d*-values are the same. Chaoite cannot be confused with nontronite or quartz or any mixture of these.

In our work only single-crystal patterns are used to identify carbynes. Single-crystal patterns give, in addition to *d*-values and reflection intensity, pattern symmetry and reflection indexing. Because of its crystal habit, chaoite gives *c*-axis patterns readily. These patterns show hexagonal symmetry and a well-established distribution of reflection intensities; moreover, the reflections obey the rhombohedral condition $-h + k + \ell = 3n$ (*8*). Nontronite is monoclinic; hence the patterns of reflections must show monoclinic symmetry. A *c*-axis pattern of nontronite appears to be hexagonal, but the distribution of intensities of the reflections is not like that of chaoite and the indexing of reflections is quite different. The *c*-axis pattern for α quartz is hexagonal, but the distribution of intensities is considerably different from those of chaoite and the reflections do not obey the rhombohedral condition. In our studies on carbynes a few very good mimics for chaoite have been found, but some feature of their diffraction data made it possible to detect the mimic.

Heimann *et al.* reported finding a linear relation between published unit cell parameters and carbyne chain length (*9, 10*). It would be very unlikely that such a relation could be obtained from a random sampling of sheet silicates. These investigators also used analytical methods similar to those of Smith and Buseck (*1*). They were able to show (*9, 10*) that it is easy to obtain misleading analytical results if there is surface contamination on a particle. Hence, this analytical method is not entirely reliable.

In view of the above, it is fair to say that there is an increasing body of evidence showing that the carbyne form of carbon does indeed exist. Moreover, the conclusions of Smith and Buseck are based on an inadequate use of diffraction data and an uncertain analytical method.

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1 June 1982; accepted 11 December 1984

Whittaker's comment on our report (*1*) raises a number of points that require response.

First, Whittaker states that the existence of triple-bonded forms of elemental carbon was established "by definition" by the laser-Raman measurements of Nakamizo *et al.* (*2*). This somewhat overstates the case put forward by Nakamizo *et al.*, who observed a weak, broad Raman band in a region considered to be characteristic of polyynes. The association of this Raman band with triple-bonded carbon by these investigators rested in part on earlier data reported by Whittaker and Kintner (*3*), so there is a risk of circular reasoning here. The laser-Raman data may perhaps be consistent with triple-bonded carbon, but these data could hardly be said to establish its existence to the point where further scientific questioning is ruled out, as implied by Whittaker.

Whittaker invokes the large body of published data on carbynes as further support for their existence. The study of something by many investigators (*4*), Russian or otherwise, provides no more compelling evidence that it exists in the form specified by Whittaker than the hundreds of papers on polywater proved its existence (*5*).

Whittaker places great significance on an infrared (IR) spectrum (given as figure 1 in his comment). However, like the electron-diffraction data of Whittaker, discussed below, interpretation of this spectrum is open to dispute. The 4.3-μm IR peak is in the correct region for –C≡C– stretching of alkyne molecules (for example, HC≡CH), which could arise by reaction with hydrogen during quenching of the carbon gas. Whittaker suggests that the 3.4-μm peak is close to the 3.1-μm value calculated for a carbyne chain (*6*). However, the difference between 3.4 μm (2940 cm^{-1}) and 3.1 μm (3230 cm^{-1}) is quite large. Moreover, the calculated position of the 3.1-μm peak depends in part on data of Whittaker and is based on a relatively primitive linear ball-and-spring model that hardly provides strong support for the interpretation of the 3.4-μm IR peak as proof of the existence of carbyne.

Even if we assume that there is an equivalence between the 3.1- and 3.4-μm values, there are problems in the interpretation of the data. If the above assignments are correct, the material contains both isolated molecules and long chains, a postulation not in accord with the proposed structure of Whittaker for carbyne (*7*). However, other interpretations are also possible for the IR data. For example, 2325 cm^{-1} (4.3 μm) corresponds to the strong, fundamental vibration of molecular CO_2 (*7*), and perhaps results from atmospheric CO_2 adsorbed on the sample; moreover, 2940 cm^{-1} (3.4 μm) is the accepted region for C–H stretching [in $-CH_3-$ or $-CH_2-$ groups (*8*)]. The broad band at 3500 cm^{-1} could result from OH stretching, perhaps from adsorbed water (*8, 9*). The IR spectrum in figure 1 of Whittaker also shows strong bands near 1750 and 1200 cm^{-1}. These are, in fact, the major peaks in the spectrum, and neither of these bands correspond to any known carbon polymorphs, even those proposed to contain carbyne (*2*). They remain to be interpreted.

We had suggested (*1*) that electron diffraction data from a chaoite sample that we examined could result from graphite with minor nontronite. Whittaker questions the absence of the nontronite 001 reflection in our table 1 (*1*). As we pointed out, the strong preferred orientation of nontronite yielded only *hk*0 powder rings; thus, the 001 reflection would not be expected. Moreover, even were it to be produced, this reflection would be hidden in the strong 000 diffraction disk. We did not consider the diffracted intensities because these are greatly affected by dynamical electron diffraction and are therefore of extremely limited use for purposes of identification.

Whittaker next stresses the importance of single-crystal electron diffraction, a technique that we indeed used for three of the four samples that we considered, including the "carbyne" grains identified and supplied to us by Whittaker. Monoclinic sheet silicates can give rise to single-crystal, electron-diffraction patterns with hexagonal symmetry (including intensities) (*10*). Two "carbyne"

grains supplied by Whittaker gave electron-diffraction patterns [and energy-dispersive, x-ray emission spectra (EDS)] indistinguishable from those obtained from talc (*1*). These crystals could not be spurious contaminants as they were identified in micrographs provided to us by Whittaker. Incidentally, the observation of Whittaker that *c*-axis patterns from chaoite show rhombohedral symmetry suggests an incomplete understanding of diffraction theory. An unknown compound that has a hexagonal pattern of spots may be indexed according to either hexagonal or rhombohedral symmetry, depending on the assumed orientation of the a^* and b^* axes.

Finally, Whittaker mentions the recent work of Heimann *et al.* (*11, 12*). The approximately linear relation found by these investigators between the crystallographic *c* parameter and the chain length for seven carbynes is to be expected, since the chain length is inferred from the *c* parameter in the first place. The scatter in this plot is then reduced by arbitrarily assigning the seven carbynes to two classes (cumulene and polyyne), giving three points on each of two lines and one point midway between them. The relationship between the *a* parameter and chain length appears to be little better than random.

The suggestion that surface contamination could account for our observed EDS is apparently based on a mistaken interpretation of our work. Heimann *et al.* (*11*) reported that we found only small amounts of silicon, aluminum, and iron, implying that this was the case for all samples studied. However, such low concentrations apply only to the nontronite-graphite intergrowth, where the nontronite is a minor component; for the other three samples that we described, the EDS signals were very strong and could not have arisen from minor surface contaminants. We remain confident of our identification of sheet-silicate minerals in these samples by means of EDS and single-crystal electron diffraction. We find nothing new in this technical comment, and we see no reason to doubt the data and interpretations in our 1982 report.

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13. We thank P. McMillan for helpful comments regarding the Raman and infrared spectral data. Support was provided by NASA grant NAG 9-59.

29 March 1985

Graft Compatibility and Clonal Identity in Invertebrates

Neigel and Schmahl (*1*) reported that morphological variation among conspecifics of the tropical sponge *Aplysina fistularis* is probably due to phenotypic plasticity (or somatic mutation) rather than to genetic variation for these traits. To determine the genetic identity of individuals, they used histocompatibility assays. They assumed that sexually produced individuals, because of genetic differences at the locus (or loci) controlling histocompatibility, were unfusible (that is, incompatible); thus, only clone mates were fusible. However, what little is known of the formal genetics of histocompatibility in clonal invertebrates—for example, in the colonial ascidian *Botryllus* (*2*), in the athecate hydroid *Hydractinia echinata* (*3*), and in the sponge *Ephydatia fluviatilis* (*4*)—shows that clonal identity is not a prerequisite for fusion. Therefore, in the absence of (i) transplants of the phenotypic variants into a common environment, (ii) reciprocal transplants between the source environments of the phenotypic variants, and (iii) formal genetic analysis of histocompatibility, their argument rests on the unproved assumption that graft compatibility (that is, fusibility) demonstrates clonal identity.

Verification of the assumption that only clone mates are fusible requires that clones (not just fusions and rejections) be distinguished unambiguously. On the basis of three criteria, Neigel and Schmahl suggested that histocompatibility assays between tissue grafts were reliable tests of clonal identity in sponges. First, fusions between randomly chosen individuals were rare, whereas all autografts fused. Second, the probability of fusion between individuals was inversely correlated to the distance between individuals. This correlation was presumed to arise from limited dispersal of clonal fragments from their source. Third, fusibility relationships were transitive (individual A fuses with B, B fuses with C, and C always fuses with A). From the absence of intransitivities, they concluded that complete genetic matching was a prerequisite for fusion. We believe, however, that these lines of reasoning do not adequately support the conclusion that fusibility implies clonal identity.

The reliability of the first criterion, that of rare fusibility among random grafts, has been questioned on theoretical grounds (*5*). Neigel and Schmahl infer from the paucity of fusions (that is, no fusions in 30 grafts separated by more than 2.1 m) that it is unlikely that any two sexually produced individuals will share histocompatibility determinants, and therefore be fusible. However, a single locus, complete genetic matching model (grafted individuals must hold both alleles in common to fuse), with as few as eight equally frequent alleles, produces fusion frequencies consistent with those reported by Neigel and Schmahl. Because the number of genotypes at any locus is defined by $[n(n + 1)]/2$, where n is the number of alleles, there need be only 36 histocompatibility genotypes to account for their results. Therefore, if the population contains more than 36 sexually produced individuals, it is highly probable that two of these individuals would be fusible. Unless the number of fusibility tests greatly exceeds the actual number of histocompatibility genotypes in a population, fusion frequency data alone cannot be used to support the assumption that only clonemates are fusible.

The second criterion, that of attenuated probability of fusion with increasing distance, is not met by one of the species in Neigel and Schmahl (*1*). We find no statistically significant negative trend, by parametric or nonparametric methods, of graft acceptance as a function of distance for *Aplysina cauliformis* [figure 2 in (*1*)]. Even the observations of no graft acceptances from pairs separated by more than 13 m is nonsignificant (binomial test; $P = 0.121$) given the small sample size ($n = 13$) and the low overall