dant at, or close to, the cell periphery. In morphologically differentiated cells, specific actin structures are easily recognized, mainly in the growth cones (Fig. 3B). After 1 hour of incubation with LAT-A or LAT-B at 35 ng/ml, most of the actin filaments and bundles disappear, and diffuse staining, dots, and patches become visible with pronounced actin accumulation in the edges of the processes (Fig. 3, C and D).

Treatment with latrunculins also disrupts the organization of actin in 3T3 cells. One hour after addition of LAT-A or LAT-B (350 ng/ml), most of the long actin bundles ("stress fibers") (*8*) are no longer visible, and intense labeling of actin is found in the cytoplasm or in the ruffling membranes. With lower concentrations (50 to 150 ng/ml), many cells display only partial disruption of their actin cables.

The effect of latrunculins on cell morphology and actin distribution is reversible, and within 1 hour after toxin removal, both N1E-115 and 3T3 cells regain their normal shape and actin structures.

Latrunculins did not have any obvious effects on the integrity of the microtubular network in either N1E-115 or 3T3 cells. In N1E-115 cells, dense aggregates of microtubule bundles appeared to run from the perinuclear region toward the edges of the cells and to extend throughout the processes, both in the absence and presence of latrunculins. Regrowth of microtubules after reversal of treatment with the depolymerizing agent colcemid (*9*) was also unaffected by the presence of latrunculins in N1E-115 and 3T3 cells, although the newly formed microtubular network conforms to the altered cell shape.

Our results show that the latrunculins represent a new class of highly potent compounds that disrupt microfilament organization in cultured cells. The mode of action of these unusual marine natural products is still unknown, but their effects resemble those of the mold metabolites, cytochalasins, the only class of drugs known to bind to actin filaments and to specifically disrupt microfilamentous structures (*10*). The latrunculins, however, exert their effects at concentrations 1/10 to 1/100 that of the cytochalasins (*11*). Furthermore, unlike the cytochalasins, the latrunculins do not alter the rate of polymerization of purified actin filaments (*12*), suggesting that the site of action of these two classes of drugs is different. Since the assembly state of actin can be modulated by various factors including actin-binding proteins (*13*), it would be important to determine whether the latrunculins act directly on actin or whether they affect this modulation process. In either case, the latrunculins should be of great value in elucidating the molecular mechanisms underlying the regulation of motile processes by the actin-microfilament systems.

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Clay Mineralogy of the Cretaceous-Tertiary Boundary Clay

Abstract. *Analyses of the clay mineralogy of samples from the Cretaceous-Tertiary boundary layer at four localities show that the boundary clay is neither mineralogically exotic nor distinct from locally derived clays above and below the boundary. The significant ejecta component in the clay that is predicted by the asteroid-impact scenario was not detected.*

Alvarez *et al.* (*1*) and other investigators (*2, 3*) have cited an anomalous iridium-rich layer at the Cretaceous-Tertiary (K/T) boundary as evidence for the collision of a large asteroid with the earth. The boundary, as determined from a change in planktonic fauna and flora, is commonly marked by a thin clay layer (1 cm to several 10's of centimeters thick) that is enriched in trace metals and 20 to 160 times above background levels in iridium. In their view, the boundary clay, or some fraction of it, was produced by the impact event and consists of a mixture of terrestrial ejecta and meteoritic material (*4*). Kyte *et al.* (*2*) have calculated that the inferred meteoritic component makes up 21 percent of the boundary clay layer in Denmark, 1.6 percent in Italy, and 9 percent in Spain (on a carbonate-free basis). Recent studies (*5*) suggest that the early high-speed ejecta from an asteroid impact could have a ratio of terrestrial to meteoritic components in this range, although earlier estimates (*1*) had suggested ratios of 100:1 or greater. The boundary clay layer should, therefore, contain a large fraction of material from the asteroid and target area.

Evidence to support the asteroid-impact hypothesis should be found in the mineralogy of the boundary layer. First, the boundary clay layer, especially the basal part of the boundary clay, should contain a mixture of meteoritic and terrestrial ejecta; since this material would be well mixed in the impact process, the boundary layer should be relatively homogeneous and mineralogically similar

Table 1. Description of samples; K/T, Cretaceous-Tertiary boundary; BC, boundary clay.

Sample	Locality	Description	Interval sampled
1	Nye Kløv	Marl	8.4 m below K/T
2	Nye Kløv	Limestone	0 to 2 cm below BC
3	Nye Kløv	Dark clay	Basal 2 to 3 mm of BC
4	Nye Kløv	Clay	Lowermost 2 cm of BC
5	Gubbio	Clay layer in limestone	1.73 m below BC
6	Gubbio	Clay	Within BC
7	Caravaca	Limestone	Below BC
8	Caravaca	Reddish clay	Basal 0.5 cm of BC
9	Caravaca	Clay	BC, 0.5 to 2 cm above base
10	Caravaca	Limestone	3.85 m above BC
11	El Kef	Limestone	70 cm below BC
12	El Kef	Clay	Lowermost 2 cm of BC
13	El Kef	Clay	BC, 1 to 2 cm above base
14	El Kef	Marl	37 cm above base of BC

at each locality. Second, the mineralogy of the boundary layer should have a component that is distinct from the locally derived clays (for example, clays in the limestones above and below the boundary) and might be expected to contain minerals derived from the asteroid and the impact area that are not normally found in the clay fraction of marine sedimentary rocks.

We examined the mineralogy of the clay fraction (< 2 μm) from the boundary layer, including samples of the basal few millimeters, from other clay layers near the boundary, and from the uppermost Cretaceous and lowermost Paleocene limestones in four sections—Nye Kløv, Denmark; Gubbio, Italy; Caravaca, Spain; and El Kef, Tunisia (*6*) (Table 1).

The samples were crushed in a mullite mortar, treated with 1*N* HCl to dissolve carbonate, immediately washed by centrifugation, dispersed by ultrasonic disaggregation, and peptized with ~ 0.01*M* sodium pyrophosphate. The fraction < 2 μm was isolated by centrifugation and concentrated by ultracentrifugation. Suspensions were dried on glass slides and treated with glycol by the vapor method for ~ 24 hours at 60°C. X-ray diffraction analysis was performed with a Siemens D-500 diffractometer equipped with a copper tube, 1° slits, and a graphite monochromator. Both air-dried and ethylene glycol–treated samples were analyzed.

Smectite (glycol treated) is identified on the basis of its 001 and higher order reflections (Fig. 1). Illite is manifested by the 001 reflection at $2\theta = 8.8°$, and in some samples by the 002 reflection at 17.7°. The 003 reflection is superimposed on the smectite 005 reflection and thus cannot be detected. The smectite from El Kef, Caravaca, and below the boundary layer at Nye Kløv may be partially converted to illite, based on the breadth of the 001 reflection, the high-intensity low-angle saddle at 3°, and the small displacements of the 002 and 003 reflections from the nominal Bragg positions. These criteria indicate randomly interstratified smectite-illite containing approximately 25 percent illite layers (*7*). More highly illitic, randomly interstratified smectite-illite is indicated by the low-angle scattering from the Gubbio samples.

Kaolinite shows strong 001 and 002 reflection maxima, and there are no peaks that correspond to the chlorite 001 and 003 reflections; thus the identification of kaolinite rather than chlorite, is made here. Some traces show a peak near 19.8°, and this is a general clay prism reflection that is not diagnostic for different clay species.

The clays from Caravaca (Fig. 1c) may contain palygorskite, as judged by the presence of a reflection at $2\theta \cong 8.5°$, which is stable in the air-dried and glycol-treated states. The second trace from the top in Fig. 1c (trace 9) shows an appended high resolution profile from $2\theta = 8°$ to 10°, obtained at a slow (0.2° per minute) scan rate. Distinct peaks occur at the illite and palygorskite positions, suggesting that palygorskite is present in this, and perhaps the other Caravaca samples from above and below the boundary, although identification based on a single reflection is tenuous.

Diffractograms of clays from the boundary sequence at Nye Kløv, Denmark (Fig. 1a), show that the boundary layer is pure smectite. The absence of discrete illite, normally a ubiquitous detrital phase in mudstones and limestones, suggests that the boundary layer here is a bentonite (altered volcanic ash). A marl layer 8.4 m below the boundary contains discrete illite and a mixed-layered smectite-illite which probably also represents altered volcanogenic material (*8*) that has been partially converted to illite by mild burial diagenesis (*9*). The clay fraction in the limestone immediately below the boundary layer is intermediate in mineralogy and consists of discrete illite (a small amount) and possibly bentonitic smectite.

The data for two samples from Gubbio, Italy (Fig. 1b), one of the boundary clay and one from a clay layer 1.73 m below the K/T boundary, are of somewhat poorer quality because of the small amounts of samples available to us. Both samples are high in what is probably terrestrial, detrital illite and kaolinite. A small amount of relatively illitic, randomly mixed-layered smectite-illite is suggested by the low-angle scattering. This material could represent a minor volcanogenic component that has been converted to smectite-illite by diagenesis, or it could be a terrigenous weathering product.

The clay minerals in the samples of the boundary clay, and clays above and below it from Caravaca, Spain (Fig. 1c) and El Kef, Tunisia (Fig. 1d), are similar. The major minerals present in all samples are illite, smectite-illite, and large amounts of kaolinite. The mineralogy may represent volcanic material that has been converted to smectite-illite diagenetically. Such bentonitic lithologies commonly contain abundant authigenic kaolinite (*8*), although the kaolinite could be terrigenous detritus. Palygorskite is present at Caravaca, but not at any of the other sites studied. The source of palygorskite in marine sediments is the subject of some debate (*10*); it may have a detrital or diagenetic origin.

Our analyses show that the boundary-layer clays differ considerably from place to place. At Nye Kløv, the boundary clay is probably a bentonite. At Caravaca and El Kef, it may be altered bentonitic material, but the large amounts of kaolinite suggest a composition and source different from those of the Danish sediments. The Gubbio clays contain less expandable minerals of any sort and have a composition apparently dominated by detrital illite and kaolinite.

Finally, there is no evidence for an exotic mineralogy within the boundary layers, even in the lowermost samples. No minerals that are unusual in normal depositional environments were detected in significant amounts, and the particular clay minerals present are most readily explained by conditions of local sedimentation. In general, all the clay assemblages are typical of Cretaceous marine limestones and mudrocks (*11*, *12*).

The sensitivity of the x-ray diffraction technique used is such that, in these samples, exotic mineral components would be detected if present in the amount of

~ 5 percent or more. Therefore, considering the measurements of meteoritic material reported by Kyte *et al.* (*2*) and the reported estimates of the ratio of meteoritic to terrestrial components expected in the ejecta (*5*), we should be able to detect a total ejecta component at all four localities, and a meteoritic component at all localities except Gubbio, if these are mineralogically exotic. Furthermore, if the ejecta were concentrated in the lower few millimeters of the clay layer (*4*), our basal samples would be composed almost entirely of impact-related ejecta. However, at Caravaca, the clay mineralogy of the sampled basal zone is not detectably different from that of the clays above and below it (Fig. 1c), and at Nye Kløv the mineralogy appears to be vertically gradational (Fig. 1a). If a layer of ejecta of nonindigenous composition is present at the base of the K/T boundary clay, its amount cannot be more than a few hundredths of that suggested by the impact hypothesis.

Arguments could be made for the diagenetic elimination of fine-grained nonclay minerals from the boundary clay. Unfortunately, no data are available on rates of alteration in low permeability rocks such as mudstones and marls; in the normal course of events, many igneous and metamorphic minerals do not survive weathering and transport if they are very fine grained. What can be said with confidence, however, is that the degree of diagenesis for the clays is mild. Highly smectitic smectite-illite indicates only incipient diagenetic alteration, perhaps representing maximum temperatures of 50° to 60°C, or possibly up to 100°C at Gubbio (*9*). The most defensible position, then, is to discount the importance of diagenetic effects unless there is evidence to the contrary.

A portion of any fine (< 1 μm) ejecta would probably be in the form of glass produced by shock melting and vaporization of the target area. This glassy material might be altered to a clay mineral such as smectite, which might be difficult to distinguish from the volcanogenic smectite present in the boundary sections. O'Keefe and Ahrens (*5*) calculate, however, that for an asteroid impact of 30 km/sec, most of the fine (< 1 μm) ejecta would be in the form of solid mineral or rock fragments (~ 0.1 of the asteroid mass, M_A) as compared with glass from both melt (~ 0.01 M_A) and condensed vapor (~ 0.01 M_A). This ratio suggests that glassy particles would make up only about 15 percent of the fine, globally distributed ejecta that would be present in an impact-related boundary layer.

Our results suggest that sediments at the K/T boundary contain relatively common clay minerals of local derivation and do not support the asteroid-impact hypothesis. Iridium and other trace metals are present in the boundary layer, but the paucity of data on iridium prevents us from making conclusive arguments for a terrestrial origin for this anomaly. The clay mineralogy (smectite and smectite-illite) suggests that the clays in and near the boundary layers formed by alteration of glassy volcanic ash. Extensive and intense volcanism is known to have occurred in the Late Cretaceous (*13*). Bentonites are numerous throughout the section, and the great volume of Cretaceous shale in North America contains major proportions of smectite and smectite-illite of likely volcanic origin (*11*). A single reference Goldschmidt (*14*) mentions that iridium and other trace metals are enriched in some andesitic volcanic ashes, possibly in heavy minerals that might be concentrated at the base of ash layers by differential settling. Apparently few new data on this have been accumulated in the past 30 years (*15*).

We propose that volcanic material be considered as an explanation of the geochemical anomalies described at the K/T

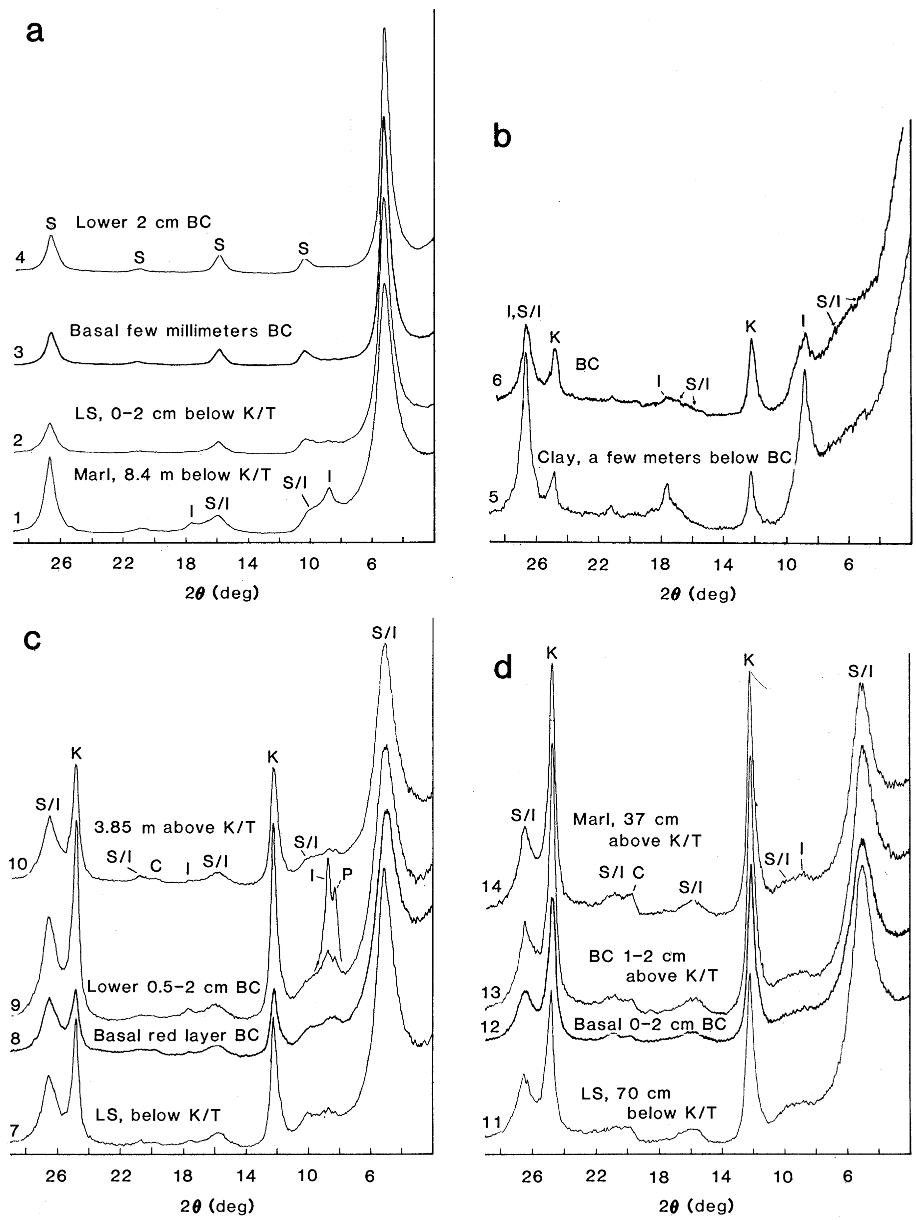

Fig. 1. X-ray diffraction patterns of samples treated with ethylene glycol; sample numbers correspond to those in Table 1, and samples from the basal part of the boundary layer are shown by heavy lines (3, 6, 8, and 12). (a) Nye Kløv, Denmark; (b) Gubbio, Italy; (c) Caravaca, Spain; a high resolution trace is shown for sample 9 from 0.5 to 2 cm above the base of the boundary clay; (d) El Kef, Tunisia. *S*, smectite; *S/I*, smectite-illite; *I*, illite; *BC*, boundary clay; *K/T*, Cretaceous-Tertiary boundary; LS, limestone; *K*, kaolinite; *P*, palygorskite; and *C*, general clay reflection.

boundary. A model that supposes an episode of intense volcanism in latest Cretaceous time would generate a rich spectrum of climatic and biological effects that would fit well into what is already known about the geologic history of that period.

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Oncogene from Human EJ Bladder Carcinoma Is Located on the Short Arm of Chromosome 11

Abstract. *The human cellular homolog of the transforming DNA sequence isolated from the bladder carcinoma cell line EJ was localized on the short arm of human chromosome 11 by Southern blot analysis of human-rodent hybrid cell DNA. This locus contains human sequences homologous to the Harvey murine sarcoma virus v-Ha-*ras *oncogene.*

Cellular transforming genes isolated from neoplasms are DNA sequences defined by their ability to induce transformation of tissue culture cells (*1*). The resulting transformed cells can be tumorigenic in nude mice (*2*). Spontaneous, chemically or virally induced neoplasms of different mammalian species contain such transforming sequences (*1–3*), some of which have been isolated from several human tumor cell lines (*4*). The oncogene of the human bladder carcinoma cell lines EJ and T24 were isolated by DNA-mediated gene transfer into NIH 3T3 mouse fibroblasts (*5–7*). These biologically active cloned transforming genes contain sequences present in normal cellular DNA. We investigated the chromosomal location of the normal sequences homologous to the transforming gene of the human EJ bladder carcinoma cell line.

We used Southern blot analysis to examine 24 different human-rodent hybrid cell lines that were derived from fusions between established Chinese hamster or mouse cell lines and normal diploid human fibroblasts or leukocytes (*8*). The hybrid cell lines retained different but overlapping complements of human chromosomes, so that any given pattern of hybridization could be unambiguously correlated with the presence of a specific human chromosome. Chromosome analysis and isozyme characterization of the hybrids was carried out at the time when DNA was extracted (*8*). Nitrocellulose filters were prepared with restriction endonuclease–cleaved DNA's from the hybrids and the parental cell lines from which they were derived. The filters were incubated with labeled probes made from clones of the biologically active 6.6-kb Bam HI fragment of the transforming gene (pEJ6.6) (*5*).

Under stringent conditions of hybridization, the pattern produced by normal human DNA is consistent with the existence of single-copy reactive sequences. A single, intense major restriction fragment was visualized in the five human donor DNA's cleaved with either Hind III or Eco RI (lanes 1 and 2 in Fig. 1A and lanes 1, 7, and 8 in Fig. 1B). Two human donor DNA's cleaved with Bam HI or Bgl II revealed the presence of two restriction fragments of the same intensity; these can be accounted for by restriction fragment length polymorphisms (lane 5 in Fig. 1C and lane 6 in Fig. 1D) (*6*). In addition to these strong signals, minor bands could also be detected in most autoradiograms. Cross-reacting sequences of varying number and intensity could be detected in Chinese hamster DNA, but they did not overlap with the human fragments detected in these blots (lane 3 in Fig. 1A and lanes 2 and 9 in Fig. 1B).

Hybrids were scored for the presence of the major fragment or the polymorphic alleles. The results (Table 1) indicate that sequences homologous to the bladder carcinoma transforming gene segregate only with human chromosome 11. The hybridization pattern was discordant with the presence of any other human chromosome in at least 40 percent of the hybrid lines. Moreover, the presence of a second closely related locus on another human chromosome could also be excluded; no positive signal in the position of the major human band was obtained in the absence of human chromosome 11 when various other human chromosomes were present. (However, parts of human chromosomes 7, 9, and 10 that were involved in complex rearrangements could not be formally ruled out.) The presence of cross-hybridizing extrachromosomal sequences is unlikely; the same hybridization pattern was observed with either nuclear DNA or total cellular DNA derived from the same hybrid line (lanes 2 and 3 in Fig. 1C).

A subset of 14 somatic cell hybrids was then used for regional localization on chromosome 11. Eight of these hybrid cell lines contained only parts of chromosome 11 (Fig. 2). The results indicated that the homologous sequences are located on the short arm of chromosome 11. Two mouse-human hybrids (series VII) had retained only one human chromosome, the result of a translocation between almost the entire short arm of chromosome 11 (region A in Fig. 2) and part of the long arm of chromosome 17 carrying the cytoplasmic thymidine kinase gene (*9*). The 6.6-kb Bam HI restriction fragment homologous to the probe is carried by this derivative chromosome, which can be selected in hypoxanthine-aminopterin-thymidine (HAT)-containing culture medium (lanes 2 and 3 in Fig. 1C) or selected against in the