

cell DNA-mediated transfection and nude mouse transformation assays (24) (Table 2). In the transfection assay no foci were observed with DNA from early passage PA1 cells under conditions that gave foci from late passage (106, 330, and 338) cell DNA. Moreover, like PA1₃₃₀-transformed NIH 3T3 cells, cells transformed with DNA from passage 106 and 338 PA1 cells have acquired the human *ras*^N sequences.

In the nude mouse transformation assay (24) DNA from early passage PA1 cells, late passage PA1 cells or human placenta was first co-transfected onto NIH 3T3 cells with the neomycin resistant plasmid pSV2-neo (25). Cells were then cultured for 2 weeks under the selective pressure of the antibiotic G418 prior to inoculation into nude mice. Of 18 nude mice, 14 developed tumors within 4 weeks after inoculation of cells receiving a late passage (338) DNA (Table 2). In contrast to the lack of focus-forming activity with the early passage cell DNA, tumors did appear in the nude mice receiving cells transfected with early passage PA1 DNA after 7 weeks; however, DNA from these tumors do not contain human *ras*^N DNA sequences. These analyses suggest that the early passage PA1 cells do not have activated *ras*^N as compared to late passage PA1 cells. This may be related to the inability of the early passage PA1 cells to clone in soft agar (data not shown) or their lack of tumorigenicity in nude mice (Table 1). Since passage 106 and 338 cells form colonies in soft agar and are tumorigenic in athymic nude mice, we can correlate tumorigenicity with the presence of the activated *ras*^N.

In addition to the activated *ras*^N, there are at least two other genetic changes occurring in PA1 cells. One is the "non-*ras*^N" dominant transforming gene in PA1₃₇ cells as observed in the occurrence of tumors in the nude mouse DNA transfection assay (Table 2). The second is a balanced translocation between chromosomes 15 and 20. Zeuthen *et al.* (15) have reported that this translocation was present at passage 224 but absent from passage 24 cells. We have observed this translocation in passage 45 and 338 PA1 cells. These genetic changes precede the appearance of the *ras*^N mutation in PA1 cells and may predispose the cell to selection of the activated *ras*^N.

There are few explanations for the appearance of the activated *ras*^N gene in late passage PA1 cells. It is possible that cells containing a mutant *ras*^N gene may have been present at low levels in the original ascites fluid. The patient with the teratocarcinoma was given chemotherapy before isolation of the ascites

fluid, and it is conceivable that such a treatment may have preferentially removed cells containing the activated *ras*^N. However, if the activated *ras*^N is sufficient to provide a selective growth advantage, those few cells in the original population would be expected to achieve predominance in the culture by passage 37. Therefore, if the mutant *ras*^N is sufficient to provide the selective advantage, it is more reasonable that the activation arose during passage in culture.

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New Radiocarbon Dates on the Cereals from Wadi Kubbania

In 1978, three carbonized grains of barley and a carbonized grain of einkorn wheat were found in a buried hearth at a Late Paleolithic site (E-78-4) at Wadi Kubbania in Egypt (1, 2). Another grain of barley was found elsewhere on the same buried living floor. In 1981, two large clusters of barley seeds, which were identified as six-row barley and thus domestic, were found at a nearby site (E-78-3) of comparable age (3). Numerous grinding stones, presumed to have been used for processing the cereals, were found in these and other sites, often deeply buried, and 30 radiocarbon dates placed the occupations between 18,500 and 17,000 radiocarbon years ago. These finds led us to suggest an early origin of food production, with implications for the initial development of complex societies (4).

Several barley seeds from site E-78-3 were analyzed by electron spin resonance spectroscopy to determine the maximal temperature to which they had been subjected before burial. A temperature of only some 150°C was indicated (5), which is too low to have brought

about the charring required for them to have survived millennia of seasonal flooding.

Therefore, six barley seeds and three small pieces of wood charcoal were dated directly by using a tandem accelerator mass spectrometer. One piece of charcoal was from site E-78-3, from a layer just above the two clusters of seeds found in 1981; the other two came from site E-78-4, from the same living-floor as the cereals. One seed was from each cluster at E-78-3, three from the group in the hearth at E-78-4, all of which had been coated with gold for scanning electron microscopy (SEM) (6), and the last, found a short distance from the hearth, had not been coated.

To make the tandem accelerator measurements shown in Table 1, the seeds were converted to carbon powder and mixed with pure iron powder. This mixture was melted to form iron-carbon beads, which were mounted on the ion source of the accelerator. The ratios of ¹⁴C/¹³C in the beads and in standard samples of iron carbide made from A.D. 1890 tree-rings were made in a manner

Table 1. Results of tandem accelerator mass spectrometer radiocarbon measurements on seeds and charcoal from Wadi Kubbania, Egypt.

Arizona number	Sample	$R\left(\frac{^{14}\text{C}/^{13}\text{C}_{\text{sample}}}{^{14}\text{C}/^{13}\text{C}_{\text{modern}}}\right)$	Radiocarbon age (years B.P.)
AA98	Barley seed, brown color, E-78-3, AF-25, 10 cm (December 1982)	0.901 ± 0.06	820 ± 500
AA97	Barley seed, black, E-78-3, AE-25, 25 cm (December 1982)	0.87 ± 0.06	1,090 ± 500
AA96	Charcoal, E-78-3, level 4 (December 1982)	0.114 ± 0.014	17,450 ± 1,000
AA226	Barley seed, E-78-4, layer a, hearth, Au-coated (April 1983)	2.39 ± 0.05	
AA225	Barley seed, E-78-4, layer a, hearth, Au-coated (April 1983)	0.723 ± 0.02	2,670 ± 250
AA227	Barley seed, E-78-4, layer a, hearth, Au-coated (April 1983)	1.327 ± 0.05	
AA228	Barley seed, E-78-4, layer a, row K-5 10 cm (April 1983)	0.551 ± 0.011	4,850 ± 200
AA224A	Charcoal, E-78-4, layer a (April 1983)	0.094 ± 0.012	19,060 ± 1,000
AA224B	Charcoal, E-78-4, layer a (April 1983)	0.107 ± 0.007	18,020 ± 525

described by Zabel *et al.* (7). The accelerator, target fabrication method, and tests have also been described by Donahue *et al.* (8). The ages given in Table 1 are radiocarbon ages calculated from a ^{14}C half-life of 5568 years, and the uncertainties are standard deviations of the average of several measurements of each quantity.

The results show that two of the three seeds that had been coated with gold had $^{14}\text{C}/^{13}\text{C}$ ratios greater than the ratio for modern material. An accelerator target made from the silver print paint used to hold the gold-coated seeds on slides for the SEM measurements proved to have a $^{14}\text{C}/^{13}\text{C}$ ratio 40 times greater than the modern ratio, indicating that results from the gold-coated seeds are not valid.

Several comments can be made concerning the other results in Table 1. First, the three measurements on charcoal agree with results obtained from conventional ^{14}C counting. Second, the two seeds from site E-78-3 could be quite recent, with radiocarbon ages of about 1000 years before present (B.P.), but these results may not be reliable since tracer ^{14}C was present in the laboratory where the SEM measurements were performed. Third, the fourth seed from site E-78-4, which was neither gold-coated nor mounted, was found to have a radiocarbon age of 4850 years B.P. ± 200 years; this is probably the true age of the cereals from site E-78-4. Although there were no indications of disturbance at the excavation site and apparently no nearby sources from which later organic remains could have been derived, the seeds are evidently intrusive.

Analyses of the Kubbania samples point up some compromises that use of the tandem accelerator mass spectrometry

in ^{14}C dating may require. Modern ^{14}C tracers are an important research tool in many botanical laboratories but may pose an unacceptable risk of contamination of archeological specimens that are to be dated. The gold-coating of specimens for SEM photography produces the best record, but it may be necessary to accept lower quality photographs if specimens are subsequently to be dated. The silver print paint used to mount specimens for SEM photography proved to be a potent contaminant.

In 1983, extensive areas of both sites E-78-3 and E-78-4 were opened and large quantities of charcoal were recovered, including numerous charred remains of plants. All the edible plants are similar to local species still growing in the area today and collected or cultivated by Egyptians (9). It has been found that the tubers of *Cyperus esculentus*, an analog of one of the more common plant remains, harden sufficiently to be ground when they have been lightly baked. This may account in part for the presence of many heavily used grinding-stones in the Kubbania sites. There were, however, no more cereals recovered. In a series of 25 soil samples from five Late Paleolithic sites in the wadi, phytoliths were present, in large quantities in some samples, but they could not be identified as those of cereals (10).

Since none of the radiocarbon dates supports the hypothesis that the cereal seeds were truly associated with the sites in which they were found, since the original cereals were not sufficiently charred to have survived for 18 millennia, since the one seed not known to have been exposed to contamination dated to 4850 year B.P. ± 200 years, since we have failed to find additional cereals

despite extensive and detailed searching, and since the sites yielded no specific cereal phytoliths, we no longer support the view that cereal use was an important component of the Late Paleolithic economy of Wadi Kubbania. Instead, the economy seems to have been one of gathering, fishing, and hunting.

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