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## Little Salt Spring, Florida: A Unique Underwater Site

A vast array of human remains, vertebrate and invertebrate fossils, and artifacts are preserved.

C. J. Clausen, A. D. Cohen, Cesare Emiliani,

J. A. Holman, J. J. Stipp

Until 1959, Little Salt Spring, located near Charlotte Harbor in southwest Florida, was thought to be one of the shallow water ponds typical of the region. Diving explorers found instead a large, flooded sinkhole some 60 meters deep. The spring consists of a basinlike depression 78 m across with its water surface 5 m above mean sea level (Fig. 1A). The floor at a rate of 42.8 liters per second. This weak flow of mineralized water is apparently a relatively recent phenomenon linked to present sea level. In the past, during periods of lower local groundwater level, the sinkhole was a freshwater cenote. As such, it attracted primitive humans in considerable numbers and for a considerable length of time.

*Summary.* Little Salt Spring in southwest Florida, consisting of a shallow, waterfilled basin above a deep, vertical underwater cavern, was a freshwater cenote in the peninsula's drier past. It collected and preserved perishable organic artifacts and other evidence of Paleo-Indian and Archaic Indian origin ranging in age from 12,000 to 9000 and from 6800 to 5200 years ago. An Archaic Period cemetery containing an estimated 1000 burials occupies an adjoining muck-filled slough and presently drowned portions of the basin of the spring. Artifacts and the nature of interment suggest a cultural link between the Archaic people and the much later Glades Tradition of southern Florida.

of the depression slopes at  $25^{\circ}$  from the land surface to 12 m in depth. A roughly circular opening 25 to 30 m across occupies the center of the depression. Below this opening, the wall is generally overhanging with two prominent encircling reentrants at depths of 18.0 and 26.0 m below the surface. The diameter of the sinkhole at its base is about 60 m, and the bottom is covered with an unknown thickness of soft detrital and organic sediments. Mineralized water, with a salinity of 3.2 per mil, a temperature of 24.4°C, and virtually no dissolved oxygen (1), flows from the cavity

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An erosional drainageway some 425 m long and 30 to 90 m wide, now a slough, leads into the basin from the northeast (Fig. 1). Postglacial, unconsolidated sediments up to a few meters thick cover the bottom of the slough, the bottom of the basin, and, to a lesser extent, the two reentrants encircling the sinkhole. These sediments, which consist of layers of detrital and organic materials including peat, contain a rich array of human remains and artifacts in an unprecedented state of preservation.

Periods of human association with the site are clearly related to lower water

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levels in the feature, which in turn reflect periods when surface water was scarce on the porous Florida carbonate platform. During periods of lower sea level associated with a cooler world climate, lower groundwater levels, lower average annual ocean (2) and land surface temperatures, and the extended effects of frontal activity (3) on peninsular weather created drier, even semiarid conditions in much of Florida. Conversely, when the water level in the feature was high, surface water was abundant everywhere and primitive humans apparently had little need for the essentially bottomless well the site represented. With the approach of sea level to its present elevation, heavily mineralized water began to issue from the feature and its usefulness to prehistoric man ended.

This is a report of our preliminary investigation of artifacts, bones, sediments, and pollen at the site. All dates are based on the carbon-14 analyses summarized in Table 1.

#### **Paleo-Indian Period**

The earliest evidence of human activity at Little Salt Spring has been found on the lower reentrant (Fig. 1A). There, the overturned, collapsed shell of an extinct species of giant land tortoise, Geochelone crassiscutata, was found with a sharply pointed wooden stake between the carapace and plastron. The orientation of this stake suggests that it entered through the exposed area between the edge of the carapace and the back of the right foreleg, piercing the pericardial or the pleuroperitoneal cavity, or both. Wood from the stake, which evidently killed the tortoise, was dated by carbon-14 at 12,030 years ago. Several of the long bones and portions of the carapace appear carbonized and numerous fragments of fire-hardened clay were found under and around the animal's remains. The tortoise was apparently killed and

C. J. Clausen is director of the Little Salt Spring Research Facility, General Development Foundation, North Port, Florida 33596. A. D. Cohen is professor in the Department of Geology, University of South Carolina, Columbia 29208. Cesare Emiliani and J. J. Stipp are professors in the Department of Geology, University of Miami, Miami, Florida 33124. J. A. Holman is professor in the Museum and Department of Geology, Michigan State University, East Lansing 48824. C. J. Clausen is senior author.







Fig. 2. (A) Broken nonreturning Paleo-Indian boomerang with cross sections. (B) Ceremonial *mutherwongal* (Smithsonian Institution specimen 392784) from North Queensland, approximately 89 cm long. (C) Boomerang (Smithsonian Institution specimen 5634) of similar length collected in Australia by the Wilks expedition (1838 to 1842). (D) Models of nonreturning boomerangs constructed of laminated wood following the style of the incomplete specimen from Little Salt Spring. Despite the difference in density between laminated wood and oak (of which the Little Salt Spring boomerang is made) the models showed excellent stability in flight and were judged fully capable of maining and downing game up to the size of small deer at a range of 40 to 60 m. Both models fractured during testing (see arrows) at the same place as the Little Salt Spring boomerang.

then cooked in situ in an upside-down position.

The presence of both loose and articulated valves of the freshwater mussel *Uniomerus obesus* (4), which existed in isolated colonies on the reentrant when the water level was only slightly higher, indicates that the water in the cavern during the cenote phase of the feature's history was fresh, although probably quite hard.

Fauna directly associated with the large tortoise includes two smaller extinct tortoises of the same species; the extinct large box turtle Terrapene carolina putnami; the extinct ground sloth Megalonyx sp.; two extant species of freshwater turtle, Chrysemys floridana and Chrysemys nelsoni; the land tortoise that presently inhabits Florida, Gopherus polyphemus; the modern diamondback rattlesnake, Crotalus adamanteus; the rabbit Sylvilagus sp.; and the wood ibis, Mycteria americana. A few meters farther east along the ledge portions of an immature mammoth or mastodon (Mammuthus or Mammut sp.) and an extinct bison were found.

By 10,000 radiocarbon years ago the water level in the feature had risen into the bottom of the basin, about 11 to 12 m below the present surface—an average rise of about 0.7 centimeter per year. During that period Paleo-Indians lived around the sinkhole. They built fires on the slope of the basin and consumed game, principally the white-tailed deer, Odocoileus virginianus. Vertebrate food refuse, together with wood, bone, shell, and stone artifacts, has been found in association with drowned informal hearths on the sand surface below more recent sediments now lining the basin. Near the bottom of the basin, around the opening to the lower cavern, numerous crudely pointed stakes made from small saplings and split segments of pine are driven into the sediment (5). The average radiocarbon age of wood from these stakes is 9572 years. Hickory nuts, isolated from the water-laid sediment into which the stakes were driven, were dated at 9920 years. Organically rich, freshwater calcitic mud samples trapped within freshwater gastropods (principally Helisoma sp.) also obtained from this sediment were analyzed for their pollen content. Although the pollen was well preserved and moderately abundant, only a relatively small number of plant types were encountered. The dominant trees in the area consisted of Myrica (wax myrtle), Quercus (oak), Pinus (pine), and Carya (hickory). The only herbaceous plants were ferns, composites, and grasses. No pollen of floating or emergent aquatic forms such as water lilies, arrowhead, or cattail was found. This suggests that the slope of the upper basin was relatively well drained and the flatland region above and surrounding the feature may still have been relatively dry (6).

Among the artifacts recovered are a socketed antler projectile point with the tip of the dart shaft still preserved in its base and the basal portion of a carved oak mortar dated at 9080 years ago, similar in style to some of those recovered from peat at the much younger (~1200 years ago) Key Marco site some 130 kilometers farther south on the coast (7).

The most important Paleo-Indian artifact so far recovered is a well-preserved portion of a nonreturning oak boomerang (Fig. 2A). This specimen displays the thinned lenticular cross section and right-angled top of some of the weapons found in Australia, particularly the swannecked or beaked variety from the Northern Territory, and should not be confused with the slightly curved, rounded "rabbit sticks" used by various American Indian groups including the Hopi of southwestern North America (8). Before the recovery of this specimen at Little Salt Spring, evidence for the use of nonreturning boomerangs was found in Australia, where the weapon type may still be in use among isolated aborigine groups (9); in ancient Egypt, where specimens of this type are reported among the grave goods of Tutankhamen (10); in India (11); and in western Europe (11). The discovery of a Paleo-Indian nonreturning boomerang in North America has a fundamental bearing on the age, origin, and distribution of this weapon (12). The specimen found at Little Salt Spring may be the oldest specimen of this type of weapon in the world and is the first found in the Western Hemisphere (13).

The water level in the spring continued to rise rapidly, averaging 0.7 cm/year, until about 8500 years ago, when it invaded the drainageway leading into the basin from the northeast and created conditions there favorable for deposition of a brown sandy peat, followed by a freshwater calcitic mud. At that time the water level of the elongated, severalhundred-meter long, standing pond was about 1.0 m below the present surface of the spring (14). The peat layer at the base of the slough contains a pollen assemblage similar to that of a modern Everglades sawgrass-water lily habitat (15). The dominant pollen represents grasses, composites, and Chenopodiaceae. These are mixed with significant amounts of Nymphaea (water lily), Typha (cattail), and Sagittaria (arrowhead). Arboreal pollen consists of small amounts of oak, pine, and willow (Salix). This wet period between 8000 and 9000 vears ago roughly corresponds to the onset of Holocene sedimentation in Mud Lake in north-central Florida and Lake Louise in the coastal plain of Georgia (16). It may also be correlated with the warmer, wetter period of mesic forest pollen evident in lake-bottom sediments in northern Georgia (17) and in the coast-

Table 1. Radiocarbon dates from Little Salt Spring and vicinity. Ages are uncorrected for reservoir or isotopic fractionation effects; their calculation is based on the Libby half-life of 5568 years. The error given is 1 standard deviation, which includes only the counting errors of the sample, modern standard, and background. All dates are years before present (1950).

Labo- ratory number	Site number	Material	Location	Depth below spring surface (m)	Depth above or below mean sea level (m)	Radiocarbon date (years before present)
Tx-2335	GDC-2120	Tortoise bone (carbonate fraction), G. crassiscutata	Reentrant at -26 m	26.0	-20.9	$13,450 \pm 190$
Tx-2636	GDF-025	Wooden stake in tortoise G. crassiscutata	Reentrant at -26 m	26.0	-20.9	$12,030 \pm 200$
I-6459	AH-14336	Peat or algal gyttja	Spring basin, below burial	7.4	- 2.3	$10,980 \pm 210$
Tx-2595	GDF-011	Charcoal (small sample) from informal hearth	Spring basin, on gray sand	~6.1	~ - 1.0	$10,190 \pm 1,450$
Tx-2461		Intact hickory nuts	Shelly calcitic mud in basin at drop-off	12.5	- 7.4	9,920 ± 160
I-6460		Wooden stake, pointed peeled sapling (formalin soak)	Spring basin at drop-off	~ 12.0	- 6.9	$9,645 \pm 160$
Tx-2460		Wooden stake, pointed split pine	Spring basin at drop-off	12.4	- 7.3	$9,500 \pm 120$
UM-1101	GDF-048	Peat or algal gyttja	Slough, below calcitic mud	N.A.†	2.9	$9.100 \pm 115$
Tx-2594	GDF-010	Wood, carved oak mortar	Spring basin on gray sand as- sociated with informal hearth	9.9	- 4.8	$9,080 \pm 250$
I-6512	UW72-1	Unidentified wood fragment	Spring basin immediately below gray sand	~ 7.7	- 2.6	8,955 ± 145
UM-1100	GDF-047	Peat or algal gyttja	Slough below burial, above calcitic mud	N.A.	3.9	8,145 ± 115
UM-1157	GDF-064	Wood, digging stick	Slough, associated with burial	N.A.	4.1	$6,830 \pm 155$
UM-1102	GDF-046	Human bone (carbonate fraction)	Slough, burial	N.A.	4.0	$6,180 \pm 95$
UM-1103	GDF-046	Human bone (collagen)	Slough, burial	N.A.	4.0	$5,850 \pm 70$
UM-1414	GDF core, 13 cm	Mucky organic deposit	Slough, core GDF-129	N.A.	4.1	5,390 ± 85
Gak-3548		Human bone (collagen)	Spring basin, burial	$\sim 8.0-9.0$	-2.0-3.0	$5,220 \pm 90$
UM-1330	GDF-125	Mucky organic deposit	Base of seasonal pond 1.8 km southeast	N.A.	~ 4.0	4,230 ± 95
UM-1412	GDF core, 7 cm	Mucky organic deposit	Slough, core GDF-129	N.A.	4.4	3,520 ± 90

\*Laboratories where the samples were radiocarbon-dated: Tx, University of Texas at Austin; I, Teledyne Isotopes, Westwood, N.J.; UM, Department of Geology, University of Miami; and Gak, Gakushuin University, Tokyo. †N.A., not applicable.

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al plain of North Carolina (18). The more abundant moisture in the drainage lows of Dismal Swamp (North Carolina) between 10,600 and 8200 years ago (19)may reflect the same wet period.

Paleo-Indian utilization of the site apparently came to an end with this high stand of water in the feature and increased availability of fresh surface water in the area.

#### **Archaic Period**

Sometime between 8500 and 8000 years ago the water level in the feature began to drop. This is evidenced by the deposition of a second layer of brown peat, the base of which, at approximately 3.9 m above mean sea level (GDF core 129), was dated at 8145 years ago. By 6800 years ago, the dropping local groundwater level and the concomitant reduced availability of surface water again made the feature attractive to humans, and Little Salt Spring became a focus of activity of Archaic Period people. Their habitation area covers 10,000 to 20,000 square meters along the higher elevations paralleling the slough, particularly along the western side, and contains well-preserved vertebrate refuse



Fig. 3. Archaic projectile points from (A) Little Salt Spring basin, (B) midden, and (C) slough. (D) Archaic projectile point from site 8-A-356 near Newnan's Lake, the type site for this point style.

(20) including undrilled shark teeth and bone, shell, and stone tools. The dead were formally interred initially in the moist, soft peat of the slough, apparently just above the changing water level of the hard-water pond. Later, as the water level in the pond continued to drop, the burial sites followed, progressing into the exposed pond basin.

The cemetery covers more than 6000  $m^2$ . The bodies were buried in extended fashion, apparently either on biers of green leafy limbs of wax myrtle, *Myrica cerifera*, or with leafy limbs placed between the arms and torso. Portions of the bodies were ceremonially wrapped with grass.

Preservation was sometimes remarkable because of the hard water and the resettling of fine peat, which combined to maintain an anaerobic environment after burial. In one case, a substantial portion of a brain with still discernible convolutions and cellular processes was found within a skull in a burial (21, 22). Dates of about 6000 years ago were obtained for human bone removed from an immediately adjacent burial. Wooden tools, including a pointed oak digging stick dated at 6830 years, and extremely well-preserved bone, shell, and stone tools have also been found with the burials. The most common artifacts are tapered points with roughly beveled bases, averaging 10 cm in length, made from the long bones of deer. The density of the burials is very high. Test explorations suggest that more than 1000 individuals rest in the cemetery. The skeletal elements of burials at the lowest elevations are equally well preserved but largely disarticulated, presumably by human foot traffic over the sloping soft sediments in which interments had been made. The associated stone projectile points are a distinctive large, triangular stemmed type known as Newnan's Lake points (Fig. 3). This type was dated at about 6000 years ago at the type site in north-central Florida and is found widely distributed over the Florida peninsula (23).

A small, incomplete wooden tablet or plaque with a raised central rib and edges bearing what may be the partial profile of a long-necked or long-billed bird (Fig. 4) was found underwater with the burials in the spring basin. This artifact resembles the typically tenoned wooden tablets or slats with carved or painted animals or geometric figures recovered in the late 1890's from the Key Marco site (24).

The Archaic component represented in and around the spring is of special importance because of the range of cultural and physical evidence present. The projectile points suggest that the Archaic inhabitants of Little Salt Spring were part of a discrete Archaic Phase, identified with Newnan's Lake points, which around 6000 years ago occupied or strongly influenced most of peninsular Florida (25). Other artifacts and the unusual mortuary practice of interring the dead with carved wooden artifacts in moist peat or muck appear to be related to the much later Glades Period culture, especially the principal sites of Key Marco farther south and Fort Center to the east on Lake Okeechobee (26). These factors suggest that the conservative, somewhat atypical nature of the Glades Tradition (27) may be attributable to compression of an Archaic culture into the southern Florida area by later, possibly agricultural, peoples entering the peninsula from the north around the time of the development and spread of ceramics, rather than to environmental forces







Fig. 4. (A) Incomplete, carved, flat wooden artifact found with Archaic burials in Little Salt Spring basin (specimen 14142, Florida State Board of Archives and History). (B) Line drawing emphasizing carved detail just above fracture. or influences from the Caribbean area. The idiosyncrasies of the Glades Tradition may reflect the effect of isolation of a strongly independent Archaic culture brought about by their hostility toward encroaching groups to the north and their containment by the sea on three sides in southern Florida (28).

The youngest radiocarbon date for a human bone specimen, which was recovered from approximately 8 to 9 m below the present water surface of the basin, is 5220 years. The average water level in the feature apparently began to rise shortly after this date, and fresh surface water (29) again became regionally abundant.

#### Conclusions

Unique cultural evidence, especially artifacts of wood, bone, and shell, which seldom survive in the Southeast, has been preserved in what can be described as a natural time capsule at Little Salt Spring. Humans inhabited the site between 12,000 and 9000 years ago and again between 6800 and 5200 years ago. During this time there were significant environmental changes related to deglaciation and the establishment of postglacial conditions. The water level in the sinkhole rose from below -26 m to within 1 m of the present surface within 3500 years (12,000 to 8500 years ago). By approximately 5500 years ago, it had dropped by about 8 m and subsequently rose to the present level (30). These changes in the water table accompanied other important environmental changes, particularly in the local flora and fauna. The paleobotanical evidence substantiates this picture, indicating a period of wetness between 9000 and 8000 years ago and again during the past 4500 years (as shown by <sup>14</sup>C dates on slough sediments). Evidence for the latter is the reappearance of such aquatic plants as water lilies, arrowhead, and cattail in the sediments of the slough (Table 1, laboratory numbers UM-1412 and UM-1414). The intervening dry periods would have exercised considerable control on human activities and the distribution of habitation sites on the inland portions of the peninsula, and this probably explains the concentration of early artifacts in solution features, spring runs, and the major drainages on the peninsula (31). The optimal humid conditions beginning around 9000 years ago may have contributed to an expanded human population. Concomitant overpredation by this population could explain the sudden disappearance of many large Pleistocene 16 FEBRUARY 1979

vertebrate species, which may have survived previous dry periods in isolated enclaves.

Little Salt Spring has already provided the earliest evidence of activity of humans in Florida, their first association with an extinct vertebrate in the Southeast, and the first evidence that they preyed on an extinct species of giant tortoise. Further investigation should provide more abundant evidence of the material culture and thus insights into the subsistence of these early people and the environment in which they lived. Information of this sort is particularly needed for the Paleo-Indian Period in the Southeast, where the subsistence pattern on the coastal plain may have been as different from that on the central and high plains of the continent (32) as the subsistence pattern in the Great Basin area has proved to be. If our projections of the number of burials in the slough and spring basin are valid, we have here the best opportunity for physical anthropological studies on an Archaic population since the discoveries decades ago at Indian Knoll, Kentucky (33).

The great wealth of human remains, artifacts, vertebrate and invertebrate fossils, and plant fossils affords a unique opportunity to reconstruct the natural and cultural environment of southwest Florida during two critical periods of recent geological history, the Pleistocene-Holocene transition and the climatic optimum. In addition, the paleontological, paleobotanical, sedimentological, mineralogical, and geochemical studies of the sediments of both the spring and the slough will provide insight into the evolution of climate during these two critical periods.

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- The possibility that nonreturning boomerangs would be discovered in a Paleo-Indian or Archa-13. The ic context in North America was anticipated by E. Callahan [M.S. thesis, Virginia Common-wealth University (1975)]. The top of a 0.85-m-thick layer of almost pure frequencies and in the above
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- and a specimen with a raised central rib. Numerous specimens of this abundant, easily recognizable point have been found in dozens of archeological sites in 59 of the 67 Florida coun-ties. Isolated specimens have been found in Ala-bama and southern Georgia. The land surface in-volved in Florida alone exceeds 100,000 km<sup>2</sup>. These points represent the most common Ar-chaic true dradged from drawed sites in Tompa chaic type dredged from drowned sites in Tampa Bay
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skeletal material was reported, the elaborately carved wooden and bone artifacts found at Key carved wooden and bone artifacts found at Key Marco in peat below muck and above a layer of presumably marine clay may now be interpreted as purposely deposited offerings rather than articles that fell into the muck from a dwelling.
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- of a number of Archaic artifact types (including various shell tools, bone points, and spear throwers) and an essentially hunting and gather-ing subsistence until the time of contact with the Europeans. The associated caramic style also Europeans. The associated ceramic style also remained essentially unchanged for more than 2000 years. According to Spanish sources, the Calusa Indians of southern Florida were almost fanatically hostile toward the agricultural tribes to the next the second to the north.
- The basement muck in a small, shallow pond 1.8 km southeast of Little Salt Spring was radio-carbon-dated at 4230  $\pm$  95 years ago (Table 1). These circular depressions containing seasonal 29 ponds are numerous on the low coastal plain north and east of Charlotte Harbor. The data reflect the onset of seasonal ponding and possibly the approach of the sea to within a few meters of its present level.

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- 33
- York, 1978)]. Clark reported some of the find-ings, including the discovery of human skeletal material, in her book *The Lady and the Sharks* (Harper & Row, New York, 1969). The site was then briefly explored by H. K. Brooks and by J.

M. Goggin [Am. Antiq. 25, 348 (1960)]. General Development Corporation (GDC) has been supporting exploratory work at the site since 1971. In 1974, GDC established the General Development Foundation, a nonprofit research organiza-tion, and later deeded to the Foundation the spring site and surrounding acreage. In addition, GDC generously provided funds to continue ex-ploration and research and has been the prime ploration and research and has been the prime contributor in the continuing investigations. Further support has been provided by the Com-mittee for Research and Exploration of the Na-tional Geographic Society; by the William G. Selby and Marie Selby Foundation of Sarasota, Florida; and by NSF grant ATM 75-22210 (Cli-mate Dynamics Program). One of us (C.J.C.) has worked at the site on eavent according a single sectors. has worked at the site on several occasions since March 1971. Scientists who have participated in March 1971. Sciencists who have participated in aspects of the exploratory work and analysis since 1971 include H. K. Brooks, H. Yezdani, C. Bramblett, H. Edgerton, W. S. Hendry, M. R. Zimmerman, H. Hollien, F. Thompson, E. Wing, J. G. Brown, M. Almy, C. Jones, and C. Peterson. Special thanks are due J. Wallace, whe provided a number of neits from the origins Peterson. Special tranks are due J. wanace, who provided a number of prints from the origi-nal glass plates of Key Marco artifacts. Con-tribution No. 11 from the Harold C. Urey Laboratory for Isotopic Paleotemperature Re-search, Department of Geology, University of Miami of Miami.

### **Total Synthesis of a Gene**

#### H. G. Khorana

Organo-chemical methods for the synthesis of oligonucleotides began to be developed (1) soon after the elucidation of the structures of the nucleic acids (2, 3). While considerable advances were made in the 1950's and 1960's in constructing polydeoxyribonucleotides of defined nu-

higher than those accessible by chemical methods are required. The formidable tasks confronting organic chemistry were recognized early (5) and, therefore, attempts were made to couple chemical methods, which alone offer oligonucleotides of controlled sequences, to other

approach enabled the preparation of a variety of double-stranded DNA-like polymers of high molecular weight and messenger RNA's (mRNA) of defined sequences (4, 6, 7). The latter proved to be very useful in studies of the genetic code. However, the large objective of the synthesis of macromolecular DNA's having nonrepeating and biologically specific sequences (5) required a different approach. Toward this goal, the central concept was the inherent ability of polynucleotide chains to form ordered bihelical complexes by virtue of base pairing. Thus, the goal was to join, end to end, chemically synthesized polydeoxyribonucleotides while these were held together in properly aligned bihelical complexes. The discoveries of the

Summary. The method developed for the total synthesis of a given DNA containing biologically specific sequences consists of the following. The DNA in the double-stranded form is carefully divided into short single-stranded segments with suitable overlaps in the complementary strands. All the segments are chemically synthesized starting with protected nucleosides and mononucleotides. The 5'-OH ends of the appropriate oligonucleotides are then phosphorylated with the use of [y-32P]ATP and polynucleotide kinase. A few to several neighboring oligonucleotides are then allowed to form bihelical complexes in aqueous solution. and the latter are joined end to end by polynucleotide ligase to form covalently linked duplexes. Subsequent head-to-tail joining of the short duplexes leads to the total DNA. The methods are described for the construction of a biologically functional suppressor transfer RNA gene. The total work involved (i) the synthesis of a 126-nucleotide-long bihelical DNA corresponding to a known precursor to the tyrosine suppressor transfer RNA, (ii) the sequencing of the promoter region and the distal region adjoining the C-C-A end, which contained a signal for the processing of the RNA transcript, (iii) total synthesis of the 207 base-pair-long DNA, which included the control elements, as well as the Eco R1 restriction endonuclease specific sequences at the two ends, and (iv) full characterization by transcription in vitro and amber suppressor activity in vivo of the synthetic gene.

cleotide sequences (4), there continued to be severe practical limits on the size of the polynucleotide chains that could be assembled unambiguously by purely chemical methods. However, for biological studies, completely defined polynucleotides in the size range often much

concepts, which together would afford high-molecular-weight nucleic acids of defined structures. Thus, in the 1960's, it was possible to use short synthetic deoxyribo-oligonucleotides with repeating sequences as templates for the nucleic acid-polymerizing enzymes, and this

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enzymes polynucleotide ligase (8) and polynucleotide kinase (9) proved crucial in these studies and, fortunately, the average size of oligonucleotides, which

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The author is Alfred P. Sloan Professor of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge 02139.