between enzymes encoded by structural genes originating from two different streptomycetes, rather than the results of the activation of latent genetic information in the recipient strains. (The latter mechanism is the most probable explanation for the three reports of the discovery of novel compounds through natural inter-strain matings (refs 18–20; discussed in ref. 21).) The evidence is most compelling for mederrhodin A, since, of the clones tested, pIJ2301, pIJ2315 and pIJ2316 (which led to mederrhodin A synthesis by AM-7161) all contain a complete transcription unit (absent from pIJ2304, pIJ2308, pIJ2312 and pIJ2317) which complements class V *act* mutants of *S. coelicolor* (F.M., unpublished); such mutants have recently been shown to be blocked in the corresponding hydroxylation involved in actinorhodin biosynthesis (S. P. Cole and H.G.F., unpublished). A true metabolic cooperation between the actinorhodin and granaticin biosynthetic enzymes is indicated also by complementation of the B1140 mutant by pIJ2308, but not by the other clones tested. B1140 can act as a secretor in co-synthesis tests with *S. coelicolor act* mutants of classes I, III and VII, but not of classes IV, V and VI[22], suggesting that its block is equivalent to that of class IV *act* mutants. Significantly, pIJ2308 is the only clone tested that carries a complete transcription unit for the class IV *act* gene (F.M., unpublished). Moreover, the 'mixed' stereochemistry found in dihydrogranatirhodin has not previously been encountered in any isochromanequinone antibiotic, even though a strain of *Streptomyces roseofulvus* has recently been found to produce both frenolicin B (with the same configuration of the pyranoid ring as actinorhodin) and nanaomycins A and $\beta$A [with the same configuration as granaticin (S.Ō. *et al.*, unpublished)].

The different results obtained with AM-7161 and Tü22 carrying the complete set of actinorhodin biosynthetic genes is interesting. In AM-7161, the donor and recipient gene sets appear to operate independently, with production of the two parental antibiotics but no novel compounds. Only when a partial *act* clone was introduced into the medermycin-producer was a hybrid product formed. In contrast, Tü22 carrying pIJ2303 produced dihydrogranatirhodin (as well as some actinorhodin), to the almost total exclusion of the normal antibiotics of the recipient. This differing behaviour may reflect several factors, including the relative specificities and affinities of the biosynthetic enzymes for their substrates, the regulation of gene expression and any possible 'channelling' of precursors within individual biosynthetic pathways. Such considerations must be borne in mind in the future use of genetic engineering in antibiotic discovery, or in attempts to modify existing antibiotics in predetermined ways.

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# Molecular cloning of Ancient Egyptian mummy DNA

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**Artificial mummification was practised in Egypt from ~2600 BC until the fourth century AD. Because of the dry Egyptian climate, however, there are also many natural mummies preserved from earlier as well as later times. To elucidate whether this unique source of ancient human remains can be used for molecular genetic analyses, 23 mummies were investigated for DNA content. One 2,400-yr-old mummy of a child was found to contain DNA that could be molecularly cloned in a plasmid vector. I report here that one such clone contains two members of the *Alu* family of human repetitive DNA sequences, as detected by DNA hybridizations and nucleotide sequencing. These analyses show that substantial pieces of mummy DNA (3.4 kilobases) can be cloned and that the DNA fragments seem to contain little or no modifications introduced postmortem.**

Samples were removed from 23 different mummies and mummy fragments. These specimens varied in age from the Sixth Dynasty (~2370–2160 BC) to late Roman times. Whenever possible, representative samples were taken from all the different tissues that could be identified visually. After rehydration and preparation of microscopical sections[1], the samples were studied using conventional histological stains as well as staining by ethidium bromide, which allows detection of small amounts of DNA. The cartilage cells from the outer ear of a mummified female head (Egyptian Museum, Berlin, GDR) as well as cells in the epidermis and subcutaneous tissues of a male head (Viktoria Museum, Uppsala; inventory number VM3251) proved to contain identifiable cell nuclei stainable by ethidium bromide. When the DNA from the latter sample was extracted, however, it was found to contain modified pyrimidines[2,3] and was intractable to molecular cloning. In contrast, cells of the epidermis and several subcutaneous structures from a less than 1-yr-old boy from the collections of the Egyptian Museum,

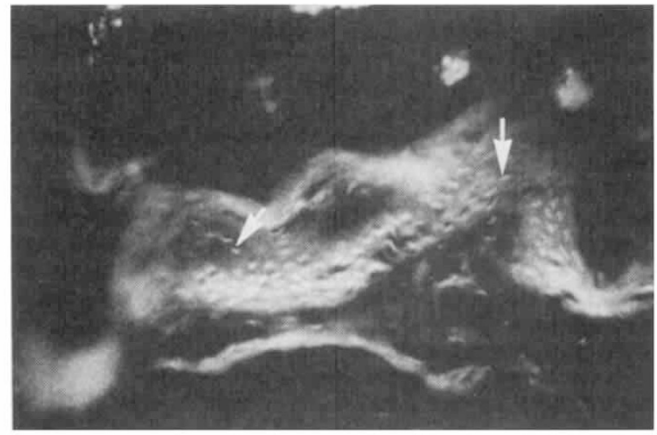

**Fig. 1** Tissue section of skin from the left lower leg of the Berlin mummy used for molecular DNA cloning. Ethidium bromide staining allows the visualization of nucleic acids in the cell nuclei (arrows).
**Methods.** Small tissue samples were rehydrated in an aqueous solution of 1% $Na_2CO_3$ (w/v), 0.5% formalin and 28.5% ethanol[1] for 48 h. Paraffin embedding and sectioning were performed using routine protocols. After deparaffination, the sections were stained in a 5 $\mu g\ ml^{-1}$ solution of ethidium bromide in phosphate-buffered saline (PBS) for 30 min, followed by extensive washing in PBS and inspection under a Leitz fluorescence microscope. ×180.

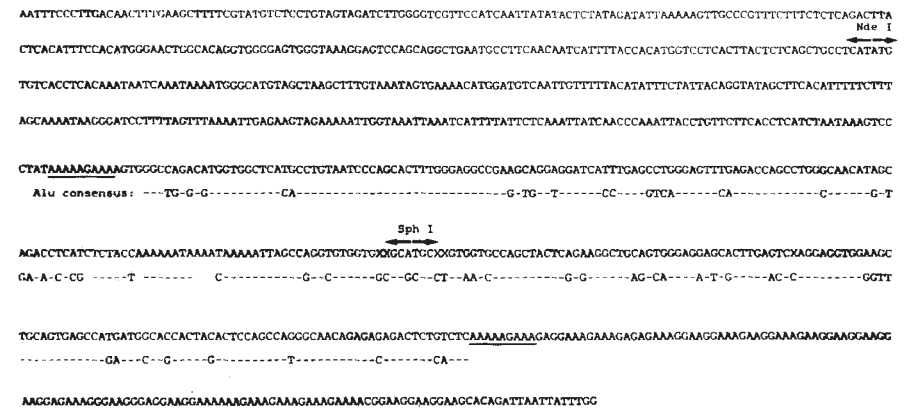


**Fig. 2** DNA sequence of part of the mummy clone pMUM2:9. The 9-bp direct repeats flanking the *Alu* sequence are underlined. In the *Alu* consensus sequence[8] only nucleotides differing from the pMUM2:9 sequence are specified.

**Methods.** DNA was prepared from 1.6 g mummy tissue, essentially according to the protocol described previously[5]. The DNA was made blunt-ended with *Escherichia coli* DNA polymerase (Klenow fragment) (Pharmacia P-L Biochemicals) according to the manufacturer's instructions and in the presence of trace amounts of radioactively labelled nucleotides. The sample was then size-fractionated by gel filtration on a G-50 column (Pharmacia, Uppsala). Excluded fractions were pooled and ethanol-precipitated and 25 ng of this material cloned in a *Sma*I-digested and alkaline phosphatase-treated pUC8 plasmid[6]. Then, 700 of the white clones were transferred to nitrocellulose filters and screened[20] with a nick-translated[7] 550-bp *BglII/SphI* fragment from a HLA-DR pseudogene[21], which contains an *Alu* repeat. The strongly hybridizing clone pMUM2:9 was isolated and restriction-mapped. Two *Alu* repeats were identified by Southern hybridization[9]. One of the *Alu* repeats as well as 500 bp of flanking DNA were sequenced according to the Maxam and Gilbert procedure[22] after labelling of the *Sph*I and *Nde*I restriction sites indicated.

Berlin, GDR (inventory no. 721) were preserved excellently by the above-mentioned criteria (Fig. 1). The radiocarbon age of this mummy was determined to 2,430 ± 120 BP, by the technique of accelerator mass spectroscopy[4].

I removed 1.6 g dry tissue from superficial parts of the left lower leg of the mummy and extracted DNA using detergent and sodium perchlorate[5] followed by phenol extraction and ethanol precipitation. When analysed by agarose gel electrophoresis, most of the DNA was <500 base pairs (bp) long. A minor portion of the material, however, was clearly ≥5,000 bp long (data not shown). Next, 15% of the extracted material was blunt-ended with *Escherichia coli* DNA polymerase (Klenow fragment) in the presence of trace amounts of radioactively labelled nucleotides. After termination of the reaction, the sample was size-fractionated by gel filtration and fractions containing the larger DNA fragments pooled and precipitated with ethanol. Then, 0.5% of this DNA was cloned in the bacterial plasmid pUC8 (ref. 6) and ~1,000 clones were obtained, 700 of which were transferred to nitrocellulose filters. Replicas of these were screened by colony hybridization using a radioactively labelled probe[7] containing a member of the *Alu* family of repetitive human sequences. The *Alu* repeats are present in at least 300,000 copies per human genome[8]. One clone (pMUM2:9), with an insert of 3.4 kilobases (kb), was identified and subjected to further analysis. On mapping by Southern hybridization[9], the insert of this clone was found to contain two different segments hybridizing to the *Alu* probe. I sequenced one of these segments and 500 bp of flanking DNA (Fig. 2). The *Alu* repeat displays 77% homology to a human *Alu* consensus sequence[8] and is flanked by 9-bp-long direct repeats. On its 3′ side it is followed immediately by a dAMP-rich region of almost 100 nucleotides. Of the point mutations present in the sequence, only 30% represent transversions, in agreement with the types of divergence found among *Alu* repeats[10]. Therefore, no significant postmortem modifications seem to have affected the nucleotide sequences analysed, even if chemical sequence alterations affecting a very small proportion of the nucleotides cannot be excluded. The DNA sequences flanking the *Alu* repeat show no statistically significant homology to any known DNA sequences contained in a DNA sequence bank[11].

These results establish the feasibility of faithfully cloning substantial pieces of genomic DNA from biological remains of great antiquity. The yield of DNA from this unusually well-preserved mummy was ~20 μg per g dried tissue, which approaches 5% of the amount expected from fresh tissue. Note, however, that most of the mummy samples investigated seem to be devoid of nucleic acids. Interestingly, the general histological preservation of the various mummy tissues was much better in superficial tissues and peripheral parts of the bodies than in the more deeply situated tissues, an observation made also by others[12,13]. This probably results from the fact that the mummification process in Ancient Egypt consisted of dehydration of the eviscerated body by embedding it in crystalline salts (natron)[14]. Thus, the parts of the body in direct contact with the dehydrating agent may have dried out first, thus shortening the time that hydrolytic processes acted upon macromolecules in these tissues. Future attempts to clone genes from Ancient Egyptian human remains should therefore be directed towards superficial tissues.

The present report, as well as the recent cloning of mitochondrial DNA sequences from the dried skin of a quagga, extinct for ~100 years[15], raise the hope that recombinant DNA techniques may be applied systematically to archaeological samples. Thus, Ancient Egyptian mummies represent a unique material that could allow diachronic studies of the frequencies of polymorphic genes in the Egyptian population since neolithic times, as well as study of the evolution of DNA-containing viruses over the same time period. Finally, a number of Egyptological problems can now be approached, such as the descent of the ancient population in the Nile valley (see ref. 16 for review) and relationships between Pharaonic dynasties as well as individual relationships between members of the Pharaonic families[17–19].

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