conclude that the CSA of certain human cells is common to VEA of GALV, SSV, and baboon endogenous type C virus.

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Phylogeny of DNA Polymerase-β

Abstract. *Analyses of various organisms for DNA polymerase-β activity show that the enzyme is widely distributed in cells from multicellular animals but absent in bacteria, plants, and protozoa. These results suggest that DNA polymerase-β may have evolved with the development of metazoan forms. Further evolutionary changes of the enzyme protein may account for some of the minor differences in properties of the enzyme in various organisms.*

DNA polymerase-β is a low molecular weight ($\leq$ 50,000), *N*-ethylmaleimide–resistant species of DNA-dependent DNA polymerase (*1*). It is widely distributed in higher vertebrate species (*2*). Its presence appears not to be related to the proliferative state of a cell or to any specific function of tissues (*2*). To ascertain whether DNA polymerase-β is some vestigial form of a primitive replicative enzyme or whether it evolved with development of new functions, I have carried out a phylogenetic survey for this enzyme activity in organisms covering a wide range of evolutionary time. The results demonstrate the widespread occurrence of this type of enzyme in multicellular animals. A comparable enzyme was not found in unicellular eukaryotes or in plants.

The operating conditions were established at the outset. Two essential properties defined for DNA polymerase-β are resistance to *N*-ethylmaleimide and low molecular weight ($\leq$ 50,000) (*1*). The *N*-ethylmaleimide resistance of the enzyme is tested by assaying enzyme activity remaining after treatment of the crude extracts (or sucrose gradient fractions) with 10 m*M* *N*-ethylmaleimide at 4°C for 30 minutes. The molecular weight of the enzyme was estimated roughly by centrifugation of the extracts on linear (5 to 20 percent by weight) sucrose gradients in 0.5*M* NaCl, 0.05*M* tris-HCl at *p*H 7.8, 1 m*M* EDTA, and 1 m*M* 2-mercaptoethanol. Centrifugation was carried out for 16 hours at 40,000 rev/min (SW 50.1 rotor; Spinco centrifuge). Two other conditions, established as optimal for mammalian enzymes, were also used to distinguish DNA polymerase-α and -β activities (*2*). The DNA polymerase-β activity was assayed with 0.05*M* Ammediol hydrochloride buffer (*p*H 8.6) and 80 m*M* NaCl; the DNA polymerase-α was assayed with 0.025*M* potassium phosphate (*p*H 7.2) and either no NaCl (for the crude extract) or 40 m*M* NaCl (for the sucrose gradient fraction). The other components in the DNA polymerase reactions were 8 m*M* $MgCl_2$, 0.1 m*M* deoxyadenosine triphosphate, 0.1 m*M* deoxycytidine triphosphate, 0.1 m*M* deoxyguanosine triphosphate, 0.1 m*M* [methyl-^{3}H]deoxythymidine triphosphate, 2 m*M* adenosine triphosphate, and 200 μg of activated calf thymus DNA per milliliter.

In interpreting the results of this survey (Table 1), the following conditions were applied. If an enzyme activity was of low molecular weight and *N*-ethylmaleimide–resistant, it was scored as DNA polymerase-β, although it is obvious that not all such activity would be absolutely identical in organisms over such a wide evolutionary scale. If an activity was of high molecular weight (> 100,000) and *N*-ethylmaleimide–resistant, it was considered outside the realm of eukaryotic enzymes, possibly of prokaryotic origin [like *Escherichia coli*

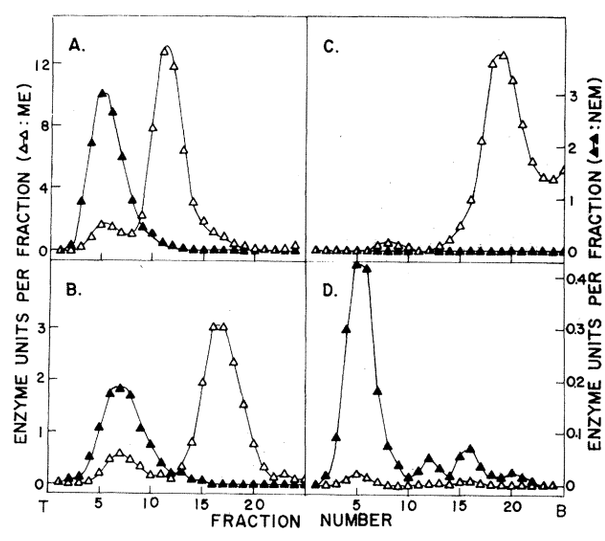

Fig. 1. Sucrose gradient analysis of DNA polymerases in (A) chameleon testes, (B) earthworm gonads, (C) silk gland, and (D) sponge. Abbreviations: ME, enzyme activity remaining after preliminary incubation with 10 m*M* 2-mercaptoethanol; NEM, enzyme activity remaining after preliminary incubation with 10 m*M* *N*-ethylmaleimide.

polymerase I (*3*)], since none of the organisms analyzed were maintained under sterile conditions. If an enzyme activity was of low molecular weight and sensitive to *N*-ethylmaleimide, it was considered possibly related to DNA polymerase-β but not scored as DNA polymerase-β. If an enzyme activity was of low molecular weight and resistant to *N*-ethylmaleimide, but its activity was lower with DNA polymerase-β reaction conditions than with DNA polymerase-α reaction conditions, it was still scored as DNA polymerase-β. The sensitivity of my analyses is such that DNA polymerase activities equal to or greater than 0.1 unit per gram of tissue or per milliliter of packed cell were detectable. A plus sign in Table 1 indicates 0.1 unit or more per gram of tissue or per milliliter of packed cells, and a minus sign indicates less than 0.1 unit per gram of tissue or per milliliter of packed cells. One enzyme unit is defined as the amount of enzyme required for polymerizing 1 nmole of nucleotide per hour at 35°C.

Table 1. Survey of major DNA polymerases in the living world.

Organism (*10*)	Tissue	DNA polymerase	
		α	β
	Chordates		
Mammals (*2*)		+	+
Aves (*11*)		+	+
Amphibian: frog (*Rana pipiens*)	Gonads	+	+
Reptile: chameleon (*Anolis*)	Testes	+	+
Pisces: toadfish (*Opsanus tau*)	Gonads	+	+
	Prechordate		
Tunicate: *Ciona*	Gonads	+	+
	Annelid		
Earthworm: *Lumbricus terrestris*	Gonads	+*	+†
	Arthropods		
Insects			
Drosophila	Embryos	+	–‡
Silkworm (*Bombyx mora*)	Silk glands: 5th instar, day 3	+*	–‡
Tobacco hornworm (*Manduka*)	Embryos	+	–‡
Crustacean: Lobster (*Homarus*)	Gonads	+	+
Chelicerate: Horseshoe crab (*Limulus*)	Testes and embryos	+	+†
	Euchiurid		
Urechis (*8*)	Eggs	+	+
	Echinoderms		
Echinoid: Sea urchin (*Lytechinus pictus*) (*8*)	Eggs and embryos	+	+
Asteroid: Starfish (*Asteria*)	Embryos and testes	+*	+
	Bryozoan		
Bugula	Whole organism	+	+†
	Mollusks		
Cephalopod: squid (*Loligo peleei*)	Testes	+*	+
Pelecypod: scallop (*Aequipecten*)	Gonads	+*	+†
	Coelenterates		
Hydrozoan: *Hydra aligactis*	Whole organism	+	+
Scyphozoan: jellyfish (*Aurelia*)	Gonads	+	+
	Platyhelminthes		
Turbellarian: *Planeria*	Whole organism	+*	+
	Porifera		
Sponge: *Microciona*	Cell suspension	–§	+
	Protozoa		
Flagellate: *Euglena gracilis* (*6*)		+	–
Ciliate: *Tetrahymena pyriformis* (*7*)		+	–
	Eubacteria		
Escherichia coli (*8*)		–‖	–
	Fungi		
Yeast: *Saccharomyces cerevisiae* (*12*)		+	–
Slime molds			
Dictyostelium discoideum	Vegetative cells and spores	+	–
Physarum polycephalum (*5*)	Microplasmodium	+	–
	Plants		
Wheat germ		+	–
Vinca rosea (*9*)		+	–

*Higher molecular weight than vertebrate DNA polymerase-α. †Low molecular weight, *N*-ethylmaleimide–resistant enzyme, but the activity is lower when assayed under the conditions for DNA polymerase-β than for DNA polymerase-α. ‡Low molecular weight enzyme but sensitive to *N*-ethylmaleimide. §The high molecular weight DNA polymerase activities resistant to *N*-ethylmaleimide are possibly of prokaryotic origin. This is probably due to contamination of the sponge cell suspension with bacteria. ‖DNA polymerase-α is defined as eukaryotic high molecular weight DNA polymerase.

Finally, certain other technical aspects were invariant. In order to avoid difficulties in interpretation of results that might occur from the use of specially differentiated tissues (for example, silk gland), embryonic or reproductive tissues were used whenever possible. Cells, tissues, or whole organisms were broken by homogenization with a glass homogenizer, sonication, or grinding with sand in a mortar and pestle in 0.2*M* potassium phosphate buffer (*p*H 7.4), 4 m*M* EDTA, and 1 m*M* 2-mercaptoethanol. When whole organisms or tissues from organisms with high levels of proteolytic activity were used, they were washed with 2 m*M* phenylmethylsulfonyl fluoride, 4 m*M* EDTA, 0.15*M* NaCl, and 0.015*M* potassium phosphate (*p*H 7.5); the washed organisms were lysed in 1 m*M* phenylmethylsulfonyl fluoride, 500 units of Trasylol per milliliter, 0.2*M* potassium phosphate (*p*H 7.5), 1 m*M* 2-mercaptoethanol, and 4 m*M* EDTA. All lysates were centrifuged for 1 hour at 45,000 rev/min (Ti 50 rotor; Spinco centrifuge). Portions of each extract were incubated with 10 m*M* *N*-ethylmaleimide or 10 m*M* 2-mercaptoethanol and assayed for DNA polymerase activities. The remainder of the extracts were dialyzed into sucrose gradient buffer and analyzed on sucrose gradients. The summary of my interpretation of all results is included in Table 1 and some representative gradient analyses are presented in Fig. 1.

The species of DNA polymerase present in the chameleon testes (Fig. 1) are similar to the mammalian enzymes both in sedimentation behavior and reaction properties. DNA polymerase-β is also found in all organisms listed as positive from the other phyla in Table 1. Several interesting variations in sedimentation rates and reaction properties of the two major DNA polymerases are seen in some of the organisms examined. For example, the DNA polymerase-β of earthworm gonads is much less active when assayed under the conditions for DNA polymerase-β than for DNA polymerase-α (Fig. 1B; note the scale difference). Further examination of this enzyme similar to DNA polymerase-β suggests a neutral *p*H optimum and only partial sensitivity to 10 m*M* *N*-ethylmaleimide. The DNA polymerase-α of earthworms also seems to have a higher molecular weight than the corresponding enzyme in vertebrates.

Major variations in the two major DNA polymerases are observed in the Arthropoda. While both enzymes found in lobster ovaries seem to be similar to the verte-

brate enzymes, the DNA polymerases of the insects vary. The DNA polymerase-α in the silk glands of silkworms seems to be a great deal larger than the vertebrate enzyme (Fig. 1C), whereas the *Drosophila* embryo DNA polymerase-α is about the same size as the vertebrate enzyme. No *N*-ethylmaleimide–resistant DNA polymerase activity is found in insect tissue extracts. However, there is a low molecular weight, *N*-ethylmaleimide–sensitive DNA polymerase activity amounting to about 1 percent of the total enzyme activity in silk gland extract and about 3 percent of total enzyme activity in *Drosophila* embryo extract. Due to the conditions set for scoring an enzyme as DNA polymerase-β, the low molecular weight enzyme from insects must be scored as negative although it may well be related to the DNA polymerase-β in other eukaryotic organisms. Mixing of silk gland extract and purified calf liver DNA polymerase-β (*4*) prior to dialysis and gradient centrifugation results in complete recovery of calf liver DNA polymerase-β activity in the gradient fraction, indicating that DNA polymerase-β–like activity is not preferentially destroyed in the extract. DNA polymerase-β activity is found in horseshoe crab testes and embryos, but its properties resemble more closely those described for the earthworm. Analysis of DNA polymerase activities in sponge, the simplest of the multicellular animals surveyed here, show DNA polymerase-β activity identical in properties to those found in the vertebrates (Fig. 1D).

The widespread occurrence of DNA polymerase-β in mammals has been noted before (*2*). In an earlier analysis of several fungi (*5*), we were unable to find any activity resembling DNA polymerase-β. No DNA polymerase-β has been reported in protozoa such as *Euglena gracilis* (*6*) and *Tetrahymena pyriformis* (*7*). The enzyme also appears to be absent in fresh extracts of log phase *E. coli* (*8*) cells. There is some controversy about DNA polymerase-β in plant cells, but my analysis of wheat germ and a study of *Vinca rosea* (*9*) indicate little or no activity of this type. The area of principal interest for this discussion then spans the region between the sponges and mammals. DNA polymerase-β is commonly found in representatives throughout this region of biological evolution.

The analyses presented certainly cannot be said to represent all living organisms, and the conditions set for analysis and interpretation are major limitations on detailed arguments about relatedness of any variants noted. Nevertheless, the presence of a well-defined DNA polymerase-β in sponge supports the hypothesis that DNA polymerase-β evolved at the time that animal cells developed a multicellular form. Subsequent evolutionary changes could account for some of the variations in reaction properties observed. The in vivo role of this enzyme in DNA synthesis and DNA repair remains unsolved. Physiological studies have shown that the level of the enzyme remains constant throughout the life cycle of a cell (*2*). The constitutive presence of the enzyme suggests that it has a maintenance function in cellular DNA metabolism. It is of interest that DNA polymerase-β–like enzyme has been conserved over half a billion years of biological evolution, from sponge to man. This observation suggests that the enzyme performs an important function in multicellular animal cells.

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Sperm Diaphorase: Genetic Polymorphism of a Sperm-Specific Enzyme in Man

Abstract. *Human sperm contains an enzyme with diaphorase activity that appears to be unique to sperm. Electrophoretic analysis of the diaphorase activity in sperm of different individuals reveals three phenotypic patterns. This polymorphism can be explained in terms of two alleles segregating at an autosomal locus; the allele frequencies have been determined to be 0.71 and 0.29. This appears to be the first reported example of a sperm-specific genetic polymorphism in man; its existence raises a number of genetic and biochemical questions.*

Many blood proteins are genetically polymorphic (*1*); less is known about the extent and expression of genetic variation in tissues other than blood. In the course of a study on the expression of genetic variation in human semen, we have discovered an enzyme with diaphorase activity that appears to be both genetically polymorphic and specific to sperm.

The sperm diaphorase polymorphism is readily detected by electrophoresis on polyacrylamide gels (Fig. 1). Sperm extracts from all individuals so far tested exhibit one of the three patterns of diaphorase activity shown in Fig. 1; the pattern type of an individual is persistent over time. The pattern designated type 1 contains a pair of enzyme bands, a and c; pattern type 2 contains bands b and d. All four bands, a, b, c, and d, are present in the pattern type designated 2-1. The composite nature of the type 2-1 pattern was indicated in an experiment in which electrophoresis of a mixture of type 1 and type 2 extracts yielded a pattern indistinguishable from the type 2-1 pattern. These sperm diaphorase patterns are not affected by varying the concentration of sperm extract applied to the gel, nor are they affected by treatment of the sperm extract with reducing agents such as mercaptoethanol.

The individual variation in the electrophoretic patterns of sperm diaphorase is very similar to the electrophoretic patterns given by other enzymes known to be genetically polymorphic (*1*). For example, in the polymorphism at the phosphoglucomutase-1 locus (PGM_1), both homozygote patterns exhibit a pair of enzyme bands, and the PGM_1 heterozygote pattern is the composite of the two homozygote patterns (*2*). By analogy, the electrophoretic variation of sperm diaphorase (SD) may be explained in terms of two allelic genes, SD^1 and SD^2, such that the homozygotes, SD^1SD^1 and SD^2SD^2, give rise to the type 1 and type 2 patterns, respectively; and the SD^1SD^2 heterozygote gives rise to the type 2-1 pattern. The full expression of heterozygosity in males would indicate that the SD locus is autosomal.

Direct demonstration of the heritable basis of this variation has not been achieved because of the difficulty of performing family studies. However, the dis-