# Reports

## Tandem Regions of Yeast DNA Topoisomerase II Share Homology with Different Subunits of Bacterial Gyrase

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**The nucleotide sequence for the *Saccharomyces cerevisiae* gene TOP2, which encodes DNA topoisomerase II, was compared with the sequence for bacterial DNA gyrase. The amino and carboxyl terminal halves of the single-subunit yeast enzyme showed homologies with the B and A subunits of bacterial gyrase, respectively, at corresponding positions along the polypeptide chains. Although the two enzymes differ in both quaternary structure and activity, the homology between the two proteins indicates mechanistic as well as structural similarities, and a probable evolutionary relationship.**

DNA TOPOISOMERASES ARE ENzymes that alter the topological state of DNA (reviewed in *1, 2*). Type II topoisomerases accomplish this by coupling ATP hydrolysis to the passage of a duplex DNA through a transient, enzyme-bridged, double-stranded break in the DNA backbone. Only one such type II DNA topoisomerase has been found in each eubacterial and eukaryotic species studied: bacterial DNA gyrase in the former and eukaryotic DNA topoisomerase II in the latter. Studies with mutants and specific inhibitors show that these type II enzymes are essential for cell viability (*2, 3*). In eukaryotes, the type II topoisomerase has been found to be a major component of the nuclear matrix and the mitotic chromosomal scaffold (*4*), leading to suggestions for a structural role of the enzyme in chromosomal organization in addition to its catalysis of DNA topoisomerization. Type II topoisomerases are also of interest because they have been shown or implicated to be targets of a number of clinically important antibiotics and antitumor drugs (*5*).

Bacterial gyrase and eukaryotic topoisomerase II show a number of characteristic differences. The bacterial enzyme contains two subunits in an $A_2B_2$ quaternary structure (*1, 2, 6, 7*), whereas the eukaryotic enzyme contains a single subunit, and is most likely a homodimer in solution (*8*). Antibiotics that are potent inhibitors of bacterial gyrase, such as nalidixic acid, oxolinic acid, and novobiocin, are ineffective or effective only at much higher concentrations when incubated with the eukaryotic enzyme (*2, 6, 7*). Finally, bacterial gyrase can catalyze the negative supercoiling of DNA, whereas eukaryotic DNA topoisomerase II can only relax negatively or positively supercoiled DNA.

We have recently sequenced the *Saccharomyces cerevisiae* gene TOP2, which encodes DNA topoisomerase II (*9*). Biochemically, yeast DNA topoisomerase II is similar to the enzyme purified from other eukaryotes (*10*). Furthermore, antitumor drugs act on the yeast enzyme in a way analogous to their effect on mammalian topoisomerase II (*11*). Given the differences between the bacterial and eukaryotic type II topoisomerases described above, it is interesting to find that tandem regions of the yeast enzyme show homology to different subunits of bacterial DNA gyrase. Figure 1 depicts the result of a homology search using the computer program of Pustel and Kafatos (*12*). The amino acid sequences of the *gyr*B and *gyr*A subunits of *Bacillus subtilis* (*13*) are displayed in tandem along the horizontal axis, with the 821–amino acid *gyr*A sequence following immediately after the 638–amino acid *gyr*B sequence. The entire 1429–amino acid sequence of DNA topoisomerase II of *S. cerevisiae* (*9*) is displayed along the vertical axis. Regions containing significant homology show a segmented diagonal line (Fig. 1). This indicates that the amino terminal half of the yeast enzyme is homologous to the B subunit of bacterial gyrase, whereas the carboxyl terminal half of the yeast enzyme is homologous to the A subunit of bacterial gyrase. Furthermore, the linear nature of the homology plot signifies that the homologies occur at corresponding positions along the polypeptide chains; that is, one does not have to add or subtract large blocks of amino acids to make the sequences align. This colinearity of the homologies strongly suggests an evolutionary and mechanistic relatedness between the two enzymes. The folding of the $NH_2$-terminal half of the yeast enzyme is presumably similar to the folding of bacterial *gyr*B, and the folding of the COOH-terminal half of the yeast enzyme presumably resembles that of bacterial *gyr*A.

Figure 2 displays the aligned yeast DNA topoisomerase II and *B. subtilis* DNA gyrase amino acid sequences. If one substitutes the *B. subtilis* gyrase A subunit sequence with the

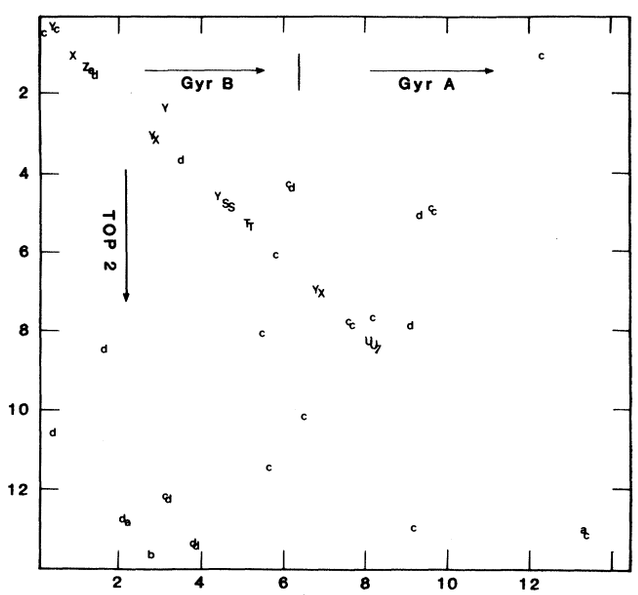


Fig. 1. Homology matrix between the amino acid sequences of DNA topoisomerase II of *S. cerevisiae* and the A and B subunit of *B. subtilis* DNA gyrase. The yeast sequence (1429 amino acids) is displayed along the vertical axis from top to bottom, and the *B. subtilis gyr*B (638 amino acids) and *gyr*A (821 amino acids) sequences are displayed in tandem along the horizontal axis from left to right. The Pustel computer program (International Biotechnologies, Inc.) (*12*) was used in the homology search; the range and scale were set at 8 and 0.90, respectively, and the capital letters A through Z in the figure signify local homology (in strings of 17 amino acids) in descending degrees ranging from 100% for A to 50% for Z; lowercase letters a through d signify local homology ranging from 49% to 42%.

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*Escherichia coli* DNA gyrase A subunit sequence (*14*), the patterns of homology are not significantly altered. Overall, the bacterial and eukaryotic type II DNA topoisomerase is 22% identical plus 14% similar in amino acids at corresponding positions. The conserved tyrosine at position 783 in yeast DNA topoisomerase II and position 123 in *B. subtilis* gyrase A subunit is probably significant. For *E. coli* gyrase, it has been shown that tyrosine-122 of the *E. coli gyr*A subunit is involved in DNA breakage and rejoining, and that it becomes covalently linked to the 5′ end of the transiently broken DNA strand (*15*). Since tyrosine-123 of the *gyr*A subunit of *B. subtilis*, which corresponds to tyrosine-122 of the *gyr*A subunit of *E. coli*, is in a region that shows homology with the yeast enzyme (Fig. 2), tyrosine-783 of yeast DNA topoisomerase II is likely to be the active site tyrosine involved in DNA topoisomerization.

Interestingly, although in *E. coli* the two genes *gyr*A and *gyr*B are widely separated on the chromosome, in *B. subtilis* they are contiguous (*13*) and the two genes are ordered in the same way as the corresponding sequences of the yeast TOP2 gene. A number of cases are known in which multiple subunits of an enzyme of one organism correspond to different domains of a single subunit enzyme of another. A well-known example is fatty acid synthetase (*16*). In the case of the type II DNA topoisomerase, it appears that gene fusion led to the covalent joining of two interactive subunits. The catalytic properties of the enzymes were significantly altered in the process, since eukaryotic DNA topoisomerase II is unable to supercoil DNA, in contrast to bacterial gyrase. In this connection, we note that a proteolytic derivative of *E. coli* gyrase missing part of the *gyr*B subunit has been observed to relax both positively and negatively supercoiled DNA but not catalyze DNA supercoiling (reviewed in *1, 2, 7*). The DNA topoisomerization activity of this derivative is no longer coupled to ATP hydrolysis, however, which is not the case with eukaryotic DNA topoisomerase II.

The relatedness between the bacterial and eukaryotic type II topoisomerases provides new insights into the mechanistic and functional properties of the enzymes. Because of the pharmacological importance of the enzymes the results should also be helpful in the search and design of new antibiotics and antitumor drugs. Finally, we note that a computer homology search (*17*) between the yeast TOP2 sequence and protein sequences other than bacterial gyrase has yielded stretches of similar regions. The region from amino acid 1200 to 1350 of yeast topoisomerase II, for example, shows

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GYRB      1                                        meqQqnsydenqiQvLEgLeLvrkrpgM
TOPOII    1  mstepvsasdkyqkislqehilkrpDtyigsvetqEqLQwJyIeetdemI

         29  yigsTnskGLhhLvwEI V dnsiDealagyctdINIqI ekDNsItYvD
          1  eknvTivpGLfkIfdEIlVnaadnNkvrdpsmkrIDVnIhaeEHtIeVkN

         77  NGRGIPVgIHeKmgrpavEVImtvLhaggkFDgsgyKVsGGlHGvGAsVv
        101  DGKGIPIeIHnKeniyipEMIfghLltssnYDddekKVtGGrNGyGAkLc

        126  NaLSTE  LDvtvhrdGkihrQtykrgVpVtdleiIgetdHtg  ttthF
        151  NiFSTEfiLEtadlnvGqkyvQkwennMsIchppkItsykKgpsytkvtF

        172  vPDpeiFsettEyDyDLLanrvReLaFltkGvnitIedkreGqerKney
        201  kPDltrFg mkElDnDILgvmrRrV YdinGsvrdInvylnGkslKirnf

        221   HYeggiksYVeyLnRsKEVvHeEpiyiegekdgItVEvalqynDsyt
        249  kNYvel   YLksLeKkRQLdNgEdgaakspiptIlIErinnrwEvafav

        268     sniySFtNnInTyeGGTHeagFkTgltrvindyarkkgLiKendpn
        296  sdisfqqiSFvNsIaTtmGGTHvn YiTdqivkkisei    LkKkkkks

        314  LsgdDVRegLtaiIsikhpDPqFegQTKtkLgnsgeRtitdtlfstamet
        341  VksfQIKnnMfifInclieNPaFtsQTKeqL    ttRvkdfgsrceiple

        364  FM  LenpDaAkKIvDkgLmaArarmAaKKarelTRRksaleisnLpgkl
        388  YInkImktDlAtRMfE  IadAneenAlKKsdg TRKsritnypkLeda

        412  adcssKDpsiseLyIvEGDSA g gsAkqgr dRhFqailPLRGKILNVe
        434  nkagtKEgykctLvLtEGDSAlslavAglavvgRdYygcyPLRGKMLNVr

        459  kArlDkILsNnEVrsMitaLGtgigedFnleKa RYhkVVIMTDaDvDGa
        484  eAsaDqILkNaEIqaIkkiMGlqhrkkYedtKslRYghLMIMTDqDhDGs

        508  HIRtLLLtFFyrymrqIIE nGYVyiaqpPLyKVqqgKrveyayndkeLe
        534  HIKgLIInFLessfpgLLDiqGFLlefitPIiKVsitKptkntiafynMp

        557  EllKtlpqtp Kpgl QRY KGLGemnAtQLwEttmdpssRtLlqvtleD
        584  DyeKwreeeshKftwkQKYyKGLGtslAqEVrEyfsnld RhLkifhslQ

        604  amDaDetfemLmgdKvEpRRnfIeanaryvknLDi
        633  gnDkDyidlaFskkKaDdRKewLrqyepgtv LDPtLKEIpISDfInkeL
GYRA      1                                    mseqntPqVREInISQeMrtsF

GYRA     23  LdYaMsviVsRaLPDVrDGLKPvHRRILYamndlgMtSDkpykksAriVg
TOPOII 682  IlFsLadnI RsIPNVlDGFKPgQRKVLYgcfkknLkSElkvaqlApyVs

         73  EvigkYHpHGDsaVyEsMVrMAQDFnyr   YMLVdgHGnFGs vdGd s
        731  Ecta YH HGEqsLaQtIIgLAQNFvgsnniYLLL pNGaFGtratGgkd

        118  AAAmRYtearMsKIsmeILRditkdtidYqDnyDgserEPv vMPsrFPn
        778  AAAaRYiyteLnKLtrkIFHpaddplykYiQe DektvEPewyLPi LPm

        167  LLVNGAaGIavGmaTnIPPhqlgEIIdgVlaVseNpDItipELmevipGp
        826  ILVNGAeGIgtGrsTyIPPfnplEIIknIrhLmnDeEL  eQMhpwfrG

        217  dfpTaGqIlgrsgIR KaYesGRgsitIrakaeIEqtssgkeRiivteL
        873   w T GtIeeiepLRyRmY  GRie qI gdnvLEitelp aRtwtstIk

        265  pYqVnkakLIekIadlvrDKkIeg ItDLrDEsDrtgMRiVIeIrrdanA
        915  eY L    LLg Lsg  nDK IkpwIkDMeEQhD dnIKfIItLspeemA

        314  nvilnnLYkqtaLqtSfgInLLaLYdgqPkvltlK qcLehyLdhQkvvI
        955  ktrkigFYerfkL iS pIsLMnMVafdPhgkikKynsVneiLs EfyyV

        363  RrrtaYElRKaEaraHiLeglRvaLDhlDaVislirnsQtaeiaRtgLie
       1002  Rl eyYQkRK D   H Mse R  LQw E V eky sfQvk fiKmiIek

        413  QfsLTeKqaqAILDmrLQrLtGLeR eKieeeYqslvkliaElkDiLaNE
       1038  EltVTnKprnAIIQe LEnL GFpRfnKegkpYygspn  dEiaEqI ND

        462  yKvleiirEEltEikeRfnDerrteIVtsglEtiedeD LIerenIvvtL
       1083  vKg atsdEE dEessH eDt  enVIngpeElygtyEyLLgm rIw sL

        511  THngYvKrLpastyrsqkrggkgVqgMgtneDdfvehListsthdtilFF
       1126  TKerYqKlL kqk qeketelenLlkLsak DiwntdLkafev gyqeFL

        561  snkgkvyRakGyeIPeyGrtaKGipiinLLEvEkgEwinaiipvtefn a
       1172  qrdaea Rg G nVPnkGsktKGkgkrkLVD D eDydpskknkkstark

        610  elyLfFttKHgvskRtsLsQfaniRnngliaLslreddeLmgvRltDgtk
       1217  gkkIkLedKN fe RilLeQklvtKskaptkIkkektpsVsetKteEeen

        660  qiiigtkngLL IRfpEtDvrEMgRtaagvKgItlTddDvVvgmeileeE
       1265  apsstssssIFdIKkeDkDegELsKisnkfKkIs TifDkMgstsatskE

        709  shvlivtEkgygKRtpaeeyRTqsRggKgLktaKitenngqLVaVkatkg
       1314  ntpeq dDva tKKnqttakKTavKp K L akKpvrkqqkVVeLsgesd

        759  eEdLmiiTasgvliR mDindIsitgRvtqgvRlirMaeeehVaTVaLvE
       1359  lE1LdsyTdredsnKdeD daIpqrsRrqrssRaasVpkksyVeTLeLsD

        808  kn  EEDEnEE   E   qEEv
       1408  dsfiEDDEeENqgsDvsfnEEd
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Fig. 2. Homology between the amino acid sequences of yeast DNA topoisomerase II and the A and B subunits of *B. subtilis*. Enumeration of all polypeptides starts with the first methionine codon whether or not the polypeptides are subject to posttranslational processing. Identical amino acids at corresponding positions are in capital letters and are underlined; amino acids that are similar at corresponding positions are in capital letters without underlining. Alignment of the sequences was either by visual inspection using the data in Fig. 1 as a guide, or by the use of a computer program of Queen and Korn (*17*). The results were substantially the same, except near the COOH-terminal portions: the computer program appears to favor COOH-terminal alignment at the expense of the overall homology. Thus, the computer output for the COOH-termini of the *gyr*B and *gyr*A sequences was amended by visual inspection to give the results shown in this figure.

many short similarities several dozen amino acids in length with histone H1 and its variants H1° and H5 (*17, 18*). These similarities typically exhibit 50% identities, and are characterized by matching lysine-rich regions. It is plausible that these similarities reflect regions in the different proteins that are involved in DNA binding. Additional examples of similarities between the yeast enzyme and other proteins include (i) weak similarity (21% identity) between a 125–amino acid region (residues 780 to 905), which includes the putative active site tyrosine at 783, and a stretch in the RNA vesicular stomatitis virus L-protein that is involved in transcription and replication (*19*); (ii) two short stretches (9 and 10 amino acids in length from positions 252 to 260 and 1039 to 1048) with 80% similarity to sequences in the type II regulatory subunit of the cyclic adenosine monophosphate–dependent protein kinase reported to contain a DNA topoisomerase activity (*20*); (iii) a 15–amino acid lysine-rich stretch (residues 327 to 340) with a 67% similarity to a region of human K-*ras* protein (*21*); and (iv) a 25–amino acid stretch with 48% identity with a region of human *myc* protein (*22*). Further biochemical and genetic information on eukaryotic DNA topoisomerase II as well as the other proteins is needed, however, to provide meaningful interpretations of these similarities.


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# The Oncogenic Activation of Human p21$^{ras}$ by a Novel Mechanism


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**Single amino acid changes were introduced into normal (non-oncogenic) and activated forms of the human H-*ras* protein at a position (residue 116) proposed on structural grounds to represent a contact site with guanine nucleotides. Substitutions at this site could significantly reduce the ability of both forms to bind and hydrolyze guanosine 5′-triphosphate; these substitutions, however, did not necessarily diminish the transforming capacity of activated derivatives. One substitution that severely impairs these functions activated the transforming potential of the otherwise normal polypeptide.**


MUTATED VERSIONS OF CELLULAR *ras* genes have been implicated in the development of many human tumors of diverse origin (*1, 2*). These genes encode a polypeptide of 21,000 daltons (p21) that is highly homologous to the transforming protein encoded by Harvey and Kirsten sarcoma viruses. Cellular and viral p21's are membrane-associated polypeptides (*3, 4*) that bind guanine nucleotides (*5*) and possess a weak guanosine 5′-triphosphate (GTP)–hydrolyzing activity (*6, 7*). It has been suggested that *ras* proteins are involved in transmembrane signal transduction—they share structural features with other signal transducers known as G proteins (*8*), and their biological activity may be similarly mediated by GTP binding (*6, 7, 9*). We wished to define residues important for GTP binding in an effort to evaluate the in vivo action of p21's with a reduced affinity for GTP. Comparative analysis of amino acid sequence has revealed several regions of homology between *ras* and other guanine nucleotide–binding proteins, including bacterial elongation and initiation factors, tubulins, members of the G-protein family, and yeast RAS proteins (*2, 8, 10*). One such region of strong homology is represented by residues 110 to 120 of p21 (*10*). This region includes Asn$^{116}$, a residue predicted on structural grounds to represent a contact site with the pyrimidine ring of guanine (*11*). To directly evaluate the role of this amino acid in the binding of p21 to GTP, we used oligonucleotide-directed mutagenesis to alter human H-*ras* complementary DNA's (cDNA's) at codon-116. We examined the ability of the variant proteins to bind and hydrolyze GTP and promote the morphological transformation of Rat-1 cells.

We replaced Asn$^{116}$ with either of two amino acids: glutamine, representing a conservative substitution, and isoleucine, which would be expected to have a more radical effect on the protein's structure. This was accomplished by converting codon-116 from AAC (asparagine) to CAG (glutamine) or to ATC (isoleucine). The alterations were detected by hybridization to specific oligonucleotide probes and verified by direct analysis of DNA sequence. The mutated cDNA's were recombined with cDNA's containing previously isolated mutations at codon-12 (*6*). In this manner we assembled cDNA's encoding p21's with glycine or valine at position 12, and asparagine, glutamine, or isoleucine at position 116. These cDNA's were introduced into *Escherichia coli* expression vectors as previously described (*6, 12*). To demonstrate the syn-


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