or the tissue is converted to a state in which treatment with a carcinogen immediately results in the formation of dormant tumor cells.

The inversion experiment thus contradicts the assumption that conversion is due to a selection and clonal expansion of neoplastic cells or to transition of initiated into dormant tumor cells. It may also be incompatible with our former interpretation that conversion is due to an expression of the neoplastic phenotype (*17*), unless TPA pretreatment creates conditions in skin that facilitate a spontaneous phenotypic expression after initation.

We would like, therefore, to modify Boutwell's concept in such a way that the process of conversion is considered as a discrete element of multistage tumorigenesis in mouse skin rather than as a component of promotion. The term "promotion" should actually be restricted to those events that must occur after carcinogen treatment and that have been called "stage II of promotion." We agree with Hennings and Yuspa (*16*) that these events may critically involve a selection and clonal expansion of tumor cells. The term "stage I of promotion" should be avoided in the future and replaced by the term "conversion," which may be operationally defined as a conversion of the tissue into a state of (increased?) promotability. This would mean that the distinction between incomplete and complete skin tumor promoters would be obsolete. Both RPA and TPA are promoters of comparable potency, but for reasons still unknown TPA is a much stronger converting agent than RPA (or mezerein). Such a reevaluation of the nomenclature is more than a matter of semantics, since it may help to overcome the discrepancy that (with few exceptions) no differences between the biological effects of RPA (or mezerein) and TPA could be found in systems other than the skin of the living mouse (*17*). Since the other systems are mainly in vitro models, the necessity of conversion might be restricted to the in vivo situation.

A mechanistic interpretation of our results is hampered by the fact that little is known about the cellular and molecular events occurring in the conversion stage. Events thought to be essential for phorbol ester–induced tumor promotion, such as an interaction with protein kinase C (*17*) and generation of superoxide anion radicals (*18*), appear not to be of critical importance for conversion. Whereas promotion is fully reversible when the intervals between individual RPA treatments are extended up to 2 weeks, conversion leads to much more persistent effects. Originally we had assumed that conversion—at least in NMRI mouse skin—is almost irreversible (*6*). We have now shown that it disappears slowly, with a half-time of approximately 10 weeks. This may be taken as an indication that long-lasting effects on epidermal cell kinetics or metabolism are involved in conversion. However, slow reversibility makes it unlikely that conversion is due to the same molecular mechanism as initiation, which is generally assumed to be irreversible. It is conceivable that—provided initiation is a rare mutagenic event—the effect leading to conversion must occur with a high degree of probability, since otherwise an inversion of the experimental sequence would not be possible.

References and Notes

1. R. K. Boutwell, *Crit. Rev. Toxicol.* **2**, 419 (1974).
2. I. Berenblum and N. Haran, *Br. J. Cancer* **9**, 268 (1955).
3. R. K. Boutwell, *Prog. Exp. Tumor Res.* **4**, 207 (1964).
4. G. Fürstenberger, D. L. Berry, B. Sorg, F. Marks, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7722 (1981).
5. T. J. Slaga, S. M. Fischer, K. Nelson, G. L. Gleason, *ibid.* **77**, 3659 (1980).
6. G. Fürstenberger, B. Sorg, F. Marks, *Science* **220**, 89 (1983).
7. A. W. Pound and J. R. Bell, *Br. J. Cancer* **16**, 690 (1962).
8. A. W. Pound, *ibid.* **22**, 533 (1968).
9. H. Shinozuka and A. C. Ritchie, *Int. J. Cancer* **2**, 77 (1967).
10. H. Hennings, G. T. Bowden, R. K. Boutwell, *Cancer Res.* **29**, 1773 (1969).
11. H. Hennings, D. Michael, E. Patterson, *ibid.* **33**, 3130 (1973).
12. T. S. Argyris, in *Carcinogenesis*, E. Hecker *et al.*, Eds. (Raven, New York, 1982), vol. 7, pp. 43–48.
13. K. Goerttler and H. Loehrke, *Exp. Pathol.* **12**, 336 (1976).
14. T. Baba, K. Aoki, M. Ishii, *Gann* **58**, 161 (1967).
15. O. H. Iversen and U. M. Iversen, *Br. J. Cancer* **45**, 912 (1982).
16. H. Hennings and S. H. Yuspa, *J. Natl. Cancer Inst.* **74**, 735 (1985).
17. F. Marks and G. Fürstenberger, in *Cellular Interactions by Environmental Tumor Promoters*, H. Fujiki *et al.*, Eds. (VNU Science Press, Utrecht, 1984), pp. 273–287.
18. M. Schwarz *et al.*, *Carcinogenesis* **5**, 1663 (1984).

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A Model for the Tertiary Structure of p21, the Product of the *ras* Oncogene

Abstract. *A model was developed for the structure of p21, the protein with a molecular weight of 21,000 that is produced by the* ras *genes. This model predicts that p21 consists of a central core of* β*-sheet structure, connected by loops and* α *helices. Four of these loops comprise the guanine nucleotide binding site. The phosphoryl binding region is made up of amino acid sequences from 10 to 16 and from 57 to 63 of p21. The latter sequence may contain a site for magnesium binding. Amino acids defining guanine specificity are Asn-116 and Asp-119, and sequences around amino acid 145 may contribute to guanine binding. The model makes it possible to visualize how oncogenic mutations of p21 affect interaction with guanine nucleotides.*

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Proteins with a molecular weight of 21,000 (p21) are produced by a family of proto-oncogenes referred to as *ras* genes. There are at least three members of the *ras* gene family in the human genome, designated H-*ras*, K-*ras*, and N-*ras* (*1*). Closely related proteins are made by the yeast *Saccharomyces*, the fruit fly *Drosophila*, and the slime mould *Dictyostelium* (*2*). Several reports have pointed to sequence homology between p21 proteins and various nucleotide-binding enzymes and regulatory proteins, such as the β-subunit of bovine mitochondrial adenosinetriphosphatase (*3*), bacterial elongation factors EF-Tu and EF-G, α-tubulin, adenylate kinase and other nucleotide-binding enzymes (*4*), and signal-transducing G proteins (*5*). Of this group, the homology between p21 and EF-Tu is particularly interesting, since EF-Tu has been extensively studied at the structural level. The primary sequence of p21 has been aligned with EF-Tu so that 42 percent of p21 amino acids have identical or conservative equivalents in EF-Tu (*6*). Further-

more, the tertiary structure of the guanosine diphosphate (GDP) binding domain of EF-Tu has recently been solved by means of x-ray crystallography (*7*). We have used this information, together with supporting biochemical, immunological, and genetic data, to generate a model for the tertiary structure of p21. This model predicts the p21 sequences responsible for guanine nucleotide specificity and for binding and hydrolysis of guanosine triphosphate (GTP), and allows us to propose mechanisms by which specific mutations may lead to oncogenic activation.

The GDP binding site of EF-Tu, analyzed at 0.29-nm resolution with x-ray crystallography (*7*), is composed of four loops; two contribute to the phosphoryl binding region and two to the guanine binding region. A magnesium ion has also been tentatively identified in the GDP binding site. These regions are shown diagrammatically in Fig. 1A. Similar regions are drawn for p21 (Fig. 1B), on the basis of sequence homologies in aligned regions of these proteins [see (*6*) and Table 1].

In EF-Tu, the phosphoryl binding region (amino acids interacting directly with the two phosphate groups of GDP) is made up of two loops, each connecting a β strand to an α helix, a structure typically seen in nucleotide binding sites. One loop is defined by the sequence Gly-His-Val-Asp-His-Gly-Lys corresponding to amino acids 18 to 24 (*7*). We propose that the equivalent loop in p21 is defined by Gly-Ala-Gly-Gly-Val-Gly-Lys, in which amino acid 12 is the second glycine residue. This assignment is based on the observation that this sequence can be aligned with the equivalent sequence of EF-Tu (*6*) and the following immunological and genetic information: First, an antibody whose epitope includes the 12th amino acid of p21 has been shown to inhibit the ability of p21 to bind GTP. Conversely, the ability of this antibody to bind to p21 is inhibited by GTP or GDP (*8*). These data have led to the proposal that this region of p21 is part of the guanine nucleotide binding site. Second, replacement of the 12th amino acid of p21 (normally glycine) with valine results in an eight- to tenfold drop in p21 guanosine triphosphatase (GTPase) activity (*9*), suggesting that sequences around position 12 interact directly with phosphoryl groups of GTP and are thus part of the GTP binding site.

Figure 2 shows how the phosphoryl binding loop of p21 may be positioned relative to the bound GDP molecule. This figure was generated by replacing EF-Tu sequence with the aligned p21 sequence and utilizes spatial coordinates

Table 1. Components of the GTP/GDP binding site from several guanine nucleotide–binding proteins.

Protein	Sequences
Phosphoryl binding sequences	
E. coli EF-Tu (*25*)	Gly His Val Asp His Gly Lys (18–24)
S. cerevisiae EF-lalpha (*26*)	Gly His Val Asp Ser Gly Lys (14–20)
Artemia (brine shrimp) EF-α (*27*)	Gly His Val Asp Ser Gly Lys (14–20)
Bovine transducin, α subunit (*9*)	Gly Ala Gly Glu Ser Gly Lys (36–42)
S. cerevisiae RAS 1 (*2*)	Gly Gly Gly Gly Val Gly Lys (17–23)
S. cerevisiae RAS 2 (*2*)	Gly Gly Gly Gly Val Gly Lys (17–23)
Human H-*ras*, N-*ras*, K-*ras* (*2*)	Gly Ala Gly Gly Val Gly Lys (10–16)
E. coli EF-Tu (*25*)	Asp Cys Pro Gly His (80–84)
S. cerevisiae EF-lalpha (*26*)	Asp Ala Pro Gly His (91–95)
Artemia (brine shrimp) EF-α (*27*)	Asp Ala Pro Gly His (91–95)
Bovine transducin, α subunit (*9*)	Asp Ser Ala Gly Tyr (145–150)
S. cerevisiae RAS 1 (*2*)	Asp Thr Ala Gly Gln (64–68)
S. cerevisiae RAS 2 (*2*)	Asp Thr Ala Gly Gln (64–68)
Human H-*ras*, N-*ras*, K-*ras* (*2*)	Asp Thr Ala Gly Gln (57–61)
Guanine specificity region	
E. coli EF-Tu (*25*)	Asn Lys Cys Asp (135–138)
S. cerevisiae EF-α (*26*)	Asn Lys Met Asp (153–156)
Artemia (brine shrimp) EF-α (*27*)	Asn Lys Met Asp (152–154)
Bovine transducin, α subunit (*9*)	Asn Lys Lys Asp (265–268)
S. cerevisiae RAS 1 (*2*)	Asn Lys Ser Asp (123–126)
S. cerevisiae RAS 2 (*2*)	Asn Lys Leu Asp (123–126)
Human H-*ras*, N-*ras*, K-*ras* (*2*)	Asn Lys Cys Asp (116–119)

obtained for EF-Tu-GDP by x-ray crystallography (*7*). In this model, the loop consisting of amino acids 10 to 16 of p21 passes under the bound guanine nucleotide close to the phosphate groups. The

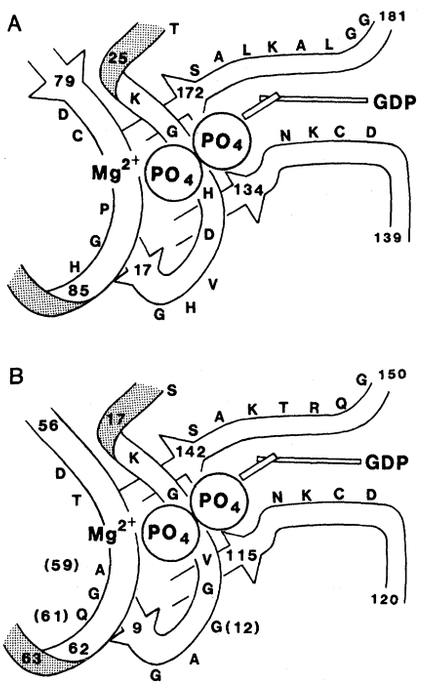

Fig. 1. Schematic representation of (A) EF-Tu and (B) p21 GDP binding sites. Amino acid sequences in the loops proposed to constitute the GDP binding sites are indicated by single letter codes: A, alanine; C, cysteine; D, aspartate; E, glutamate; G, glycine; H, histidine; K. lysine; L. leucine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; and V, valine.

distance of the backbone of the loop from the phosphates is predicted to be sufficiently close to allow hydrogen bonding to occur. The exact positioning of the p21 loop is, of course, likely to differ from that of the EF-Tu loop because the sequences are not identical.

Table 1 shows sequence alignments from amino terminal regions of several guanine nucleotide–binding proteins, including the α subunit of transducin. On the basis of the EF-Tu structure, and on less direct evidence for p21, it is probable that these related sequences are also part of the phosphoryl binding regions. Each of these loops contains glycine residues that impart a high degree of flexibility. This flexibility may be possible because the loops stand out from a rigid structure composed of β strands connected by α helices. The presence of a flexible phosphoryl binding region at the transition between a β strand and an α helix is typical of many nucleotide-binding proteins (*10*). It is thought that the α helix generates an electrical dipole with the positive end at the amino terminus (*11*): this field may have the effect of increasing the on-rate for nucleotide binding and may be involved in positioning the phosphoryl groups favorably for catalytic hydrolysis.

In EF-Tu, a loop that includes amino acids 80 to 84 has been found adjacent to the bound guanine nucleotide, as indicated diagrammatically in Fig. 1A. Figure 1B shows a similar arrangement in which aligned amino acids from p21 (*6*) are

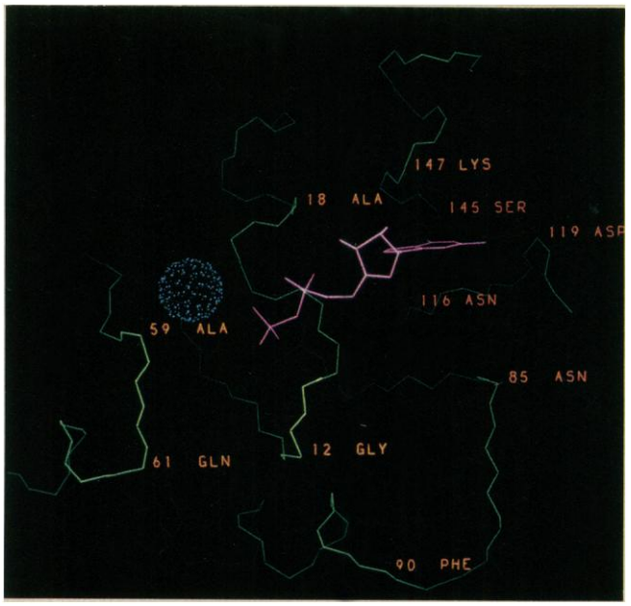

Fig. 2. Proposed spatial arrangement of p21 GDP binding site. Magnesium ion is represented as a blue sphere, GDP by pink lines, and the backbone of the polypeptide chain by green lines, the intensity of which represents the distance from the viewing surface.

substituted in this model. The predicted spatial arrangement of these amino acids is depicted in Fig. 2. It can be seen that amino acids 59 and 61, both activating positions of p21, are close to the bound nucleotide and constitute part of the nucleotide binding site. Furthermore, a magnesium ion has been tentatively identified in the guanine nucleotide binding site of EF-Tu (*7*) where it may be coordinated to aspartate-80. p21 has an aspartate at the aligned position (amino acid 57), as do the other G proteins shown in Table 1. Magnesium has been shown to be directly involved in the mechanism of GTP hydrolysis by EF-Tu (*12*). Likewise, magnesium is essential for GTP hydrolysis by p21 (*9*), and it seems likely that this ion is located at an equivalent position in this protein as shown in Fig. 2. We have found that millimolar concentrations of magnesium (or manganese) inhibit the ability of p21 to bind to antibodies that interact with the 12th amino acid (*13*). This provides supporting evidence for the proximity of bound magnesium to sequences around position 12.

In EF-Tu, two noncontiguous loops contribute to the guanine binding pocket (*7*). Both of these are loops connecting β-sheet to α-helical regions. They consist of amino acids 135 to 138 (Asn-Lys-Cys-Asp) and 173 to 176 (Ser-Ala-Leu-Lys), as shown in Fig. 1. The sequence Asn-Lys-Cys-Asp is found in p21 at amino acids 116 to 119, as others have pointed out (*4, 6*). Side groups of these amino acids define the specificity of EF-Tu for guanine nucleotides, Asp hydrogen bonding to the amino group and Asn to the keto group on the pyrimidine ring (*7*). These residues may perform the same function in p21 and in the other GTP/GDP–binding proteins listed in Table 1. In p21, replacement of Asn^{116} or Asp^{119} with Ala, by site-directed mutagenesis, eliminates GTP binding to p21 (*14*); these results are consistent with the proposal that this part of p21 interacts with guanine of GTP. In EF-Tu, Leu^{175} contributes a hydrophobic region to the guanine pocket. p21 has no leucine at an equivalent position, and it is not clear whether other aligned residues (as depicted in Fig. 1B) have any role in guanine binding.

The GDP binding domain of EF-Tu corresponds to about 50 percent of the protein's mass, or about 21,000 daltons of amino acid sequence (*7*). Since p21 is a similar size and may have a similar GDP/GTP binding site, it is possible that the tertiary structure of the EF-Tu GDP binding domain is similar to the tertiary structure of the entire p21 protein. By substituting homologous sequences of p21 in the EF-Tu model, a model for the tertiary structure of p21 can be drawn (Fig. 3). In this model, the overall structure of p21 consists of four parallel β strands connected by α helices and loops. This structural feature, the so-called Rossmann fold, has been observed in many nucleotide-binding proteins (*10*). There are no intramolecular cystine bridges in this model structure. The ease with which p21 can be renatured after complete reduction and denaturation is consistent with this suggestion (*9*). Furthermore, it has been demonstrated experimentally that EF-Tu does not contain intramolecular cystine bridges (*15*). The carboxy terminal region of p21 is not homologous to EF-Tu, and varies between different p21 proteins. No particular structure can therefore be easily assigned to this region.

The secondary structure of p21 has been predicted on the basis of the algorithms of Chou and Fasman (*16*) and Garnier (*17*). Briefly, these analyses predict each of the β strands shown in Fig. 2, and each α helix, with the exception of the first which is predicted to be a β strand (*9, 18*). Each of the regions that are proposed to be involved in GTP binding and hydrolysis (amino acids 10 to 16, 56 to 63, 116 to 119, and possibly 144 to 160) are predicted to be coils by these analyses. These results therefore lend support to the model shown in different ways in Figs. 2 and 3.

A novel family of *ras*-related genes, designated *rho* genes, has recently been described (*19*). These genes encode proteins that are about 35 percent homologous to p21. It is striking to note that conserved regions between *ras* and *rho* gene products are predominantly in the four domains proposed here to constitute the guanine nucleotide binding site; specific amino acids such as the aspartate residue at position 57 that may bind magnesium, and the asparagine and aspartate residues at 116 and 119 that confer guanine specificity, are identical in products of *rho* and *ras*.

It is evident that the colinear arrangement of phosphoryl binding regions and guanine binding regions that is shared between the proteins listed in Table 1, as well as the *rho* gene products, raises the possibility that all these proteins have similar tertiary structures, so that the structural arrangement depicted in Fig. 3 may represent the general structure of all other related G proteins.

The best characterized biochemical property of p21 is its ability to bind guanine nucleotides. The essential role of guanine nucleotide binding to p21 was demonstrated recently by Feramisco and co-workers (*20*), who microinjected an antibody that inhibits GTP binding to p21 into p21-transformed cells. Injected cells reverted transiently to an untransformed phenotype as a result of specific inactivation of the oncogene. Genetic analysis of oncogenic forms of p21 has revealed that they differ from normal, proto-oncogenic forms by single base changes, usually in the 12th or 61st codon (*1*). Both of these positions are in the guanine nucleotide binding site of p21, according to this model.

Several groups have investigated the guanine nucleotide–binding properties of purified p21, and have proposed that the biochemical basis of oncogenic activation of p21 is a reduction in the rate of hydrolysis of bound GTP by mutant

forms (*9*); this reduction in GTPase activity could maintain p21 in an "active" GTP-bound form for a longer time and so cause inappropriate cell proliferation. It is likely that the GTP-bound form of p21 differs from the GDP-bound form in its ability to interact with other cellular proteins, and that the basis of this difference may be conformational changes that accompany GTP or GDP binding. La Cour and co-workers have predicted that the conformation of the guanine nucleotide binding domain of EF-Tu is likely to be different when GTP is bound rather than GDP (*7*); this prediction was based on a consideration of atomic spacing with the use of computer graphics, which indicated that the EF-Tu loop 80 to 84, which corresponds to p21 sequences 57 to 61, would be unacceptably close to the γ-phosphate of GTP. If indeed the guanine nucleotide binding site of p21 resembles that of EF-Tu, as proposed here, the same steric constraints would apply, and an altered conformation of p21-GTP would be predicted. It is interesting that the phosphoryl binding region of EF-Tu contains valine at the position corresponding to Gly^{12} of p21 and may more closely resemble the structure of the oncogenic valine mutation.

It has been demonstrated that replacement of glycine at position 12 with any other amino acid, except proline, activates the transforming potential of p21 (*21*). This lack of selectivity for the replacing amino acid suggests a common structural difference between normal p21 and activated forms. According to our model, the 12th amino acid of p21 is in the phosphoryl binding loop (Figs. 1 to 3). It is likely that replacement of Gly^{12} with other amino acids would result in changes in the conformation of the loop, and that these changes would affect the interaction of the loop with the bound nucleotide. Glycine is unique among naturally occurring amino acids in not having a side chain; this allows a greater range of main chain dihedral angles than other amino acids. This distinction between glycine and all other amino acids may account for the observation that all substitutions at position 12, except proline, have qualitatively similar effects on the biological properties of p21. It may be significant that proline also differs from all other amino acids in generating a fixed main chain dihedral angle. This angle may be close to the angle adopted when glycine is at position 12 but different from the angle adopted when any other amino acid is at this position, so that interaction of the phosphoryl binding loop with bound nucleotide is not significantly altered by this substitution.

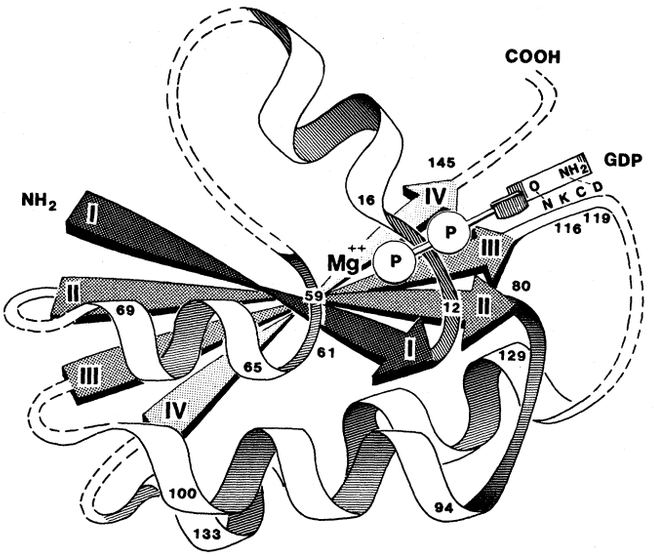

Fig. 3. Model for the tertiary structure of p21. Significant amino acids and β strands are numbered, the latter with Roman numerals. Broken lines represent regions in which sequence homology with EF-Tu is insignificant.

The acutely transforming retroviruses Harvey sarcoma virus and Kirsten sarcoma virus contain *ras* oncogenes transduced from rat cells. Both of these *ras* genes are altered at position 59 (as well as 12), each encoding threonine instead of alanine at this position. This threonine becomes the substrate for GTP-dependent autophosphorylation (*22*), the γ-phosphate being transferred to the hydroxyl group of threonine. Fig. 2 shows that Ala^{59} is adjacent to the β phosphate of GDP. The hydroxyl group may be positioned such that it replaces a water molecule normally coordinated to magnesium in the GTP binding site. Substitution of alanine for threonine reduces GTPase activity of p21 (*9*); this may be because threonine interferes directly and specifically with hydrolysis, and not because of structural effects on the positioning of β and γ phosphates, as suggested for position 12 mutations. In support of this it is interesting that all activating position 59 mutants so far detected, including those generated by random mutagenesis in vitro (*23*), are threonine substitutions. Normal p21 has a threonine at position 58; the side chain of this residue is expected to face away from the bound guanine nucleotide, by analogy with the equivalent residue of EF-Tu (Cys^{81}). This hydroxyl group is therefore not available as a substrate for autophosphorylation.

Mutations at amino acid 61 of p21 activate the oncogenic potential of p21 and reduce GTPase activity (*9*). The proposal that amino acid 61 is part of the GTP binding site is consistent with these findings and has been suggested previously (*6, 23*). However, from the model shown in Fig. 2 it is not obvious how Gln^{61}, which appears to be at the edge of the cleft defining the guanine nucleotide pocket, could interact directly with GTP or affect GTP hydrolysis.

The preceding discussion can be summarized as follows: mutations at certain positions in p21 may alter the interaction of p21 with phosphoryl groups of bound guanine nucleotide, or with magnesium and water molecules in the nucleotide binding site. These altered interactions reduce p21 GTPase activity (*9*) so that the protein remains in a GTP-bound state rather than a GDP-bound state. The essential difference between these states is conformational and affects the interaction between p21 and other cellular components. These interactions, in turn, contribute to cellular transformation. It should be noted that activating mutations in p21 could conceivably lead directly to these conformational changes, and that effects on GTP hydrolysis could be secondary to these changes.

The proposed structural similarity between the guanine nucleotide binding domains of *ras* gene products, elongation factors, and mammalian G proteins leads to the notion that these domains have a similar function, that of a biochemical switch. Whether this switch is "on" or "off" is determined by the phosphorylation state of the bound nucleotide. For example, EF-Tu · GTP binds aminoacyl-tRNA with high affinity, but this affinity decreases by two orders of magnitude when the bound nucleotide is hydrolyzed to GDP on the ribosome. This decrease is thought to be the result of a change in conformation that accompanies loss of the γ-phosphate group of GTP. EF-Tu · GDP is released from the ribosome, and can be restored to the GTP-bound form by an exchange reaction catalyzed by EF-Ts (*24*). A similar situation has been demonstrated for G proteins. GTP-bound forms of these regulatory proteins

interact with their target enzymes (adenylate cyclase or phosphodiesterase) and are converted to inactive GDP-bound forms by intrinsic GTPase activity. GTP-bound forms are regenerated by interaction with activated receptors. It is likely that, by analogy with EF-Tu and G proteins, the GTP- and GDP-bound forms of p21 represent active and inactive conformations that are interconverted by intrinsic GTPase and catalytic GTP-GDP exchange. Identification of the role of the active form of p21 and knowledge of the mechanism by which it is regenerated after hydrolysis would greatly accelerate understanding of the transformation process.

References and Notes

1. C. J. Marshall and P. W. J. Rigby, *Cancer Surv.* **3**, 183 (1984); E. Taparowsky *et al.*, *Cell* **34**, 581 (1983).
2. D. DeFeo-Jones *et al.*, *Nature (London)* **306**, 707 (1983); D. Gallwitz, C. Dorath, C. Sander, *ibid.*, p. 704 (1984); S. Powers *et al.*, *Cell* **36**, 607 (1984); B. Shilo and R. Weinberg, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6789 (1981); T. Pawson *et al.*, *Mol. Cell. Biol.* **5**, 33 (1985).
3. N. J. Gay and J. E. Walker, *Nature (London)* **301**, 262 (1983).
4. R. Leberman and U. Enger, *EMBO J.* **3**, 339 (1984).
5. J. B. Hurley *et al.*, *Science* **226**, 860 (1984); D. C. Medynski *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
6. K. Halliday, *J. Cyclic Nucleotide Protein Phosphorylation Res.* **9**, 435 (1984).
7. T. la Cour *et al.*, *EMBO J.*, in press.
8. R. Clark *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5280 (1985).
9. J. P. McGrath, D. J. Capo, D. V. Goeddel, A. D. Levinson, *Nature (London)* **310**, 646 (1984); R. W. Sweet *et al.*, *ibid.* **311**, 273 (1984); J. B. Gibbs *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5704 (1984); V. Manne, E. Bekesi, H-F. Kung, *ibid.* **82**, 376 (1985).
10. G. E. Schulz and R. H. Schirmer, in *Principles of Protein Structure* (Springer-Verlag, New York, 1978).
11. W. G. J. Hol, P. T. van Duijnen, H. J. C. Berendsen, *Nature (London)* **273**, 443 (1978).
12. A. Wittinghofer *et al.*, *Eur. J. Biochem.* **124**, 109 (1982).
13. R. Clark, G. Wong, F. McCormick, unpublished data.
14. F. McCormick, M. Trahey, G. Cole, M. Innis, in preparation.
15. R. A. Laursen *et al.*, *J. Biol. Chem.* **256**, 8102 (1981).
16. P. Y. Chou and G. D. Fasman, *Biochemistry* **13**, 222 (1974).
17. J. Garnier *et al.*, *J. Mol. Biology* **120**, 97 (1978).
18. N. Katre and F. McCormick, unpublished data.
19. P. Madaule and R. Axel, *Cell* **41**, 31 (1985).
20. J. R. Feramisco *et al.*, *Nature (London)* **314**, 639 (1985).
21. P. H. Seeburg, W. W. Colby, D. J. Capon, D. V. Goeddel, A. D. Levinson, *ibid.* **312**, 71 (1984).
22. T. Shih et al., *ibid.* **287**, 686 (1980).
23. M. Wigler *et al.*, in *Cancer Cells* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1984), pp. 419–425.
24. H. Weissbach, in *Ribosomes: Structure, Function and Genetics*, G. Chamblis, G. R. Craven, J. Davies, K. Davies, L. Kahar, M. Nomura, Eds. (University Park Press, Baltimore, 1979), pp. 377–341.
25. M. Jones *et al.*, *Eur. J. Biochem.* **108**, 507 (1980). Numbering system based on protein sequence.
26. F. van Hemelt *et al.*, *EMBO J.* **3**, 1109 (1984). Numbering system based on genomic sequence.
27. S. Nagata *et al.*, *EMBO J.* **3**, 1825 (1984). Numbering system based on genomic sequence.

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Z-DNA Forms Without an Alternating Purine-Pyrimidine Sequence in Solution

Abstract. *Nuclear magnetic resonance spectra (proton and phosphorus-31) and ultraviolet absorption spectra of the DNA decamer d(br^{5}CGbr^{5}CGATbr^{5}CGbr^{5}CG), in which the central two adenine-thymine base pairs are out of order with the rest of the purine-pyrimidine alternation sequence, indicate that under appropriate solvent conditions (high salt and methanol) the molecule undergoes a structural transition from a right-handed B-DNA conformation to a left-handed Z-DNA conformation. Measurements of the two-dimensional nuclear Overhauser effect on the decamer indicate that all of the guanines as well as the two equivalent thymines adopt the* syn *conformation.*

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There is a dynamic equilibrium between right-handed B-DNA and left-handed Z-DNA, and the former is usually the major species (*1*). In B-DNA, all bases are in the *anti* conformation, whereas in Z-DNA, consecutive bases alternate between the *anti* and *syn* conformations. Purines adopt the *syn* conformation more readily than pyrimidines; thus the B to Z equilibrium has, until recently, been studied in solution only in polymers with alternating purine-pyrimidine sequences such as poly(dG-dC) (*2, 3*), and poly [d(A-C) · d(G-T)] as well as other modified polymers (*4, 5*), or in oligomers such as (m^{5}dC-dG)$_3$ (*6, 7*). Indirect evidence has suggested that naturally occurring sequences of DNA that are not strictly alternating purine-pyrimidine can be stabilized in the Z conformation by negative supercoiling (*8*). To investigate the structure of the putative Z-DNA in such sequences, we designed a series of DNA oligomers that were brominated at the 5-position of cytosine, since previous studies had shown that this modification strongly favors the Z-DNA conformation (*9*). A recently examined crystal structure of the DNA oligomer d(br^{5}CGATbr^{5}CG) showed that it adopts the Z conformation with its thymines in the *syn* conformation (*10*), but its stability as Z-DNA in solution remains questionable.

We now show that in solution the modified DNA decamer, d(br^{5}CGbr^5-CGATbr^{5}CGbr^{5}CG), which has a nonalternating purine-pyrimidine sequence, undergoes a structural transition from the B to Z conformation under appropriate solvent conditions. Large changes in the proton (^{1}H) and phosphorus (^{31}P) nuclear magnetic resonance (NMR) spectra are observed when the solvent is changed from an aqueous solution with low salt concentration to one that contains a high salt concentration and methanol. Measurements of the two-dimensional nuclear Overhauser effect (2D-nOe) (*10, 11*) indicate that all of the guanines as well as the two equivalent thymines are in the *syn* conformation in a methanol-water solution with a high concentration of salt. The differences between the absorption spectra of the molecule in the two solvent systems are also

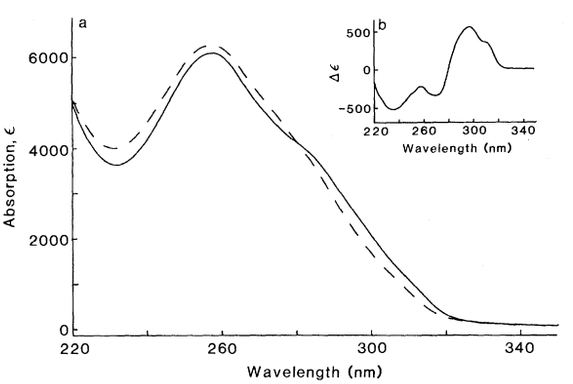

Fig. 1. (a) Ultraviolet absorption spectra of d(br^{5}CGbr^5-CGATbr^{5}CGbr^{5}CG) in aqueous solutions containing 0.1*M* NaCl (dashed line) and 3*M* NaCl and 33 percent methanol (solid line). (b) The difference spectrum (Z − B). There is an isosbestic point at about 279 nm. We also found that the absorption spectrum of the decamer in a 0.1*M* NaCl solution with 33 percent methanol is nearly identical to that in 0.1*M* NaCl alone. The DNA was prepared by improved phosphotriester synthesis (*22*). The starting material for the modified cytosines was 5-bromodeoxycytidine. The DNA concentration for these ultraviolet absorption experiments is 100 times less than for the NMR experiments.