GTP-Binding Proteins As Oncogenes in Human Tumors

by Frank McCormick*

Ras oncogenes are the most commonly known oncogenes in human tumors; their prevalence in different types of tumors and their role in the development of these tumors have been discussed in recent reviews (1,2). We have investigated the biochemical mechanism that accounts for the activation of ras proto-oncogenes to become oncogenes. In normal cells, the ras p21 cycles between the active guanosine triphosphate (GTP)-bound form and the inactive form, bound to guanosine diphosphate (GDP). The conversion of the GTP- to the GDP-bound form is catalyzed by a cellular protein referred to as GAP (GTPase activating protein) (3). The mechanism by which the GTP-bound state is generated is not known; however, this mechanism must be much slower than the GAP-catalyzed reaction, so that most of the ras p21 in normal cells is in the GDP-bound state. In ras-transformed cells, the oncogenic mutant ras protein exists in its GTP-bound state constitutively. This is because GAP fails to catalyze hydrolysis of GTP bound to these proteins. Oncogenic mutants can therefore be thought of as mutants that escape from this aspect of GAP function.

Two types of GAP cDNA have been cloned from human placenta (4). Figure 1 shows the structure of the proteins encoded by these cDNAs. Type I GAP is a 116,000 Da protein, with a hydrophobic amino terminus, two SH2 regions (src-homology region 2), one SH3 region, and a catalytic domain comprising the C-terminal one-third of the protein (5). All cells express type I GAP, whereas type II, which lacks the first 180 amino acids of type I GAP and is generated by an alternative splicing mechanism, has only been detected in human placenta and in certain human cell lines, such as MRC-5 diploid fibroblasts.

Interaction of GAP with ras p21 may have consequences additional to conversion of the GTP-bound to the GDP-bound form. This possibility was raised by the discovery that GAP binds to a region of p21 that is necessary for ras function (6,7), suggesting that GAP itself might be part of a ras effector system. This hypothesis has not yet been proved, but additional support has been provided by the observation that oncogenic mutants of ras p21 retain their ability to bind GAP (8), even though binding does not lead to GTPase activation (i.e., downregulation). To date, no ras p21 mutant has been described that is biologically active yet fails to bind GAP.

Heterotrimeric G-proteins involved in signal transduction from hormone receptors to internal second messengers cycle between GTP- and GDP-bound forms in a similar manner to ras p21 proteins. Again, the active form is the GTP-bound state. However, these proteins rely on intrinsic GTPase activity to convert active to inactive forms, rather than depending on an accessory protein such as GAP. Although the mechanism of down-regulation is therefore different, these proteins are also susceptible to constitutive activation by point mutations that lock them in the GDP-bound state. Landis and coworkers described point mutations in the G-protein alpha subunit (the GTP-binding subunit) in human pituitary tumors (9). These mutations occurred at arginine-201 or glutamine-227. Arginine-201 has no exact homolog in the ras p21 protein, although it is in a region of Gs-alpha that is equivalent to the GAP-binding region. Glutamine-227 is the equivalent of glutamine-61 in ras p21 (a frequent site of oncogenic activation). Both types of mutation reduce intrinsic GTPase of Gs-alpha, as expected for activating mutations.

To extend these observations, we have used polymerase chain reaction (PCR) to amplify genomic DNA from over 300 tumor samples and have analyzed the frequency of mutations in Gs-alpha in codons 201 and 227 (10). Mutations at these positions occurred in pituitary adenomas (43% of samples tested) and in thyroid tumors (4%). We have also initiated a survey of other G-protein alpha subunits that may be activated in hu-

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man cancers. Initially, we identified codons of Gi-2 alpha subunit that are equivalent to codons 201 and 227 of Gs alpha. We then reanalyzed the panel of 300 tumor samples for point mutations at one of these positions. Of all the tumors tested, tumors of the adrenal cortex (27%), ovarian granulosa (33%), and medullary thyroid (9%) were found to contain Gi-2 mutations, in all cases at codon 179, the Gi-2 equivalent of 201 in Gs-alpha. The substitutions produced by these mutations (arginine to histidine or cysteine) were the same as those of Gs-alpha codon 201, suggesting a common mechanism of activation.

For Gs-alpha, a major effector is known: this G-protein turns on adenylyl cyclase activity. Effector systems for Gi-2 are less clearly defined. Gi proteins were originally defined as G-proteins that inhibit adenylyl cyclase, but are now known to have other functions, which may be direct or indirect consequences of Gi activation.

In conclusion, striking parallels can be drawn between activation of ras genes in human tumors and activation of G-proteins. Both involve inhibition of GTPase activity, albeit by rather different mechanisms. As a result, the GTP-binding protein is locked in the GTP-state and signals constitutively. However, G-protein activation is so far confined to tumors of endocrine function, whereas ras mutations occur in most types of tumors. We presume that this means that ras p21 proteins activate pathways that are more intimately involved with cellular proliferation, whereas those of G-proteins are more specialized. In one case (Gs-alpha) the pathway is known, so that for the first time, an oncogene function can be understood in terms of the second messenger pathway it controls. We hope that pathways activated by ras proteins and Gi proteins will be elucidated in the near future.

REFERENCES

1. Bos, J. L. ras oncogenes in human cancer: a review. Cancer Res. 49: 4682–4688 (1989).
2. Barbacid, M. ras Genes. Annu. Rev. Biochem. 56: 779–827 (1987).
3. Trahey, M., and McCormick, F. A cytoplasmic protein stimulates normal N-ras p21 GTPase, but does not affect oncogenic mutants. Science 238: 542–545 (1987).
4. Trahey, M., Wong, G., Halenbeck, R., Rubinfeld, B., Martin, G. A., Ladner, M., Long, C. M., Crosier, W. J., Watt, K., Koths, K., and McCormick, F. Molecular cloning of two types of GAP complementary DNA from human placenta. Science 242: 1697–1700 (1988).
5. Marshall, M. S., Hill, W. S., Ng, A. S., Vogel, U. S., Schaber, M. D., Scolnick, E. M., Dixon, R. A. F., Sigal, I. S., and Gibbs, J. B. A C-terminal domain of GAP is sufficient to stimulate ras p21 GTPase activity. EMBO J. 8: 1105–1110 (1989).
6. Adari, H., Lowy, D. R., Willumsen, B. M., Der, C. J., and McCormick, F. Guanosine triphosphatase activating protein (GAP) interacts with the p21 ras effector binding domain. Science 240: 518–521 (1988).
7. Cales, C., Hancock, J. F., Marshall, C. J., and Hall, A. The cytoplasmic protein GAP is implicated as the target for regulation by the ras gene product. Nature 332: 548–551 (1988).
8. Vogel, U.S., Dixon, R. A. F., Schaber, M. D., Diehl, R. E., Marshall, M. S., Scolnick, E. M., Sigal, I. S., and Gibbs, J. B. Cloning of bovine GAP and its interaction with oncogenic ras p21. Nature 335: 90–93 (1988).
9. Landis, C. A., Masters, S. B., Spada, A., Pace, A. M., Bourne, H. R., and Vallar, L. GTPase inhibiting mutations activate the α chain of Gs, and stimulate adenylyl cyclase in human pituitary tumors. Nature 340: 692–696 (1989).
10. Lyons, J., Landis, C. A., Harsh, G., Vallar, L., Grunewald, K., Feichtinger, H., Duh, Q.-Y., Clark, D. H., Kawasaki, E., Bourne, H. R., and McCormick, F. Two G protein oncogenes in endocrine tumors. Science 249: 655–659 (1990).