Perspective

GTP-GDP Exchange Proteins

ALEXANDER LEVITZKI

RAS PROTEINS AND THEIR HOMOLOGS, COLLECTIVELY termed small molecular weight G proteins, seem to function analogously to heterotrimeric G proteins, which transduce signals from a large family of receptors to an expanding number of biochemical effectors (*1*). The most prominent member of the family of small G proteins is the mammalian c-H-*ras*–encoded p21 protein (H-Ras) and its oncogenic homolog v-H-Ras in which Gly^{12} has been mutated to Val^{12}. This mutation causes H-Ras to remain in its guanine triphosphate (GTP)–bound, active form, signaling the cell to divide continuously (*2*). GTPase activating protein (GAP) (*3*) may be a component of this effector. A parallel search for the upstream regulators of H-Ras was stimulated by the recognition that yeast RAS proteins play key biological roles in *Saccharomyces cerevisiae* (*4, 5*) and *Schizosaccharomyces pombe* (*6*). In *S. cerevisiae*, in which adenosine 3′,5′-monophosphate (cAMP) is a major signal for cell growth and division, RAS2 (and less so RAS1) activates the yeast adenylyl cyclase, the protein product of the *CDC35/CYR1* gene (*5*). H-*ras* can replace, rather effectively, endogenous yeast *RAS* genes by activating adenylyl cyclase (*7, 8*). This finding plus the observation that a mutated yeast RAS transforms NIH 3T3 cells (*8*) has spurred investigation of *S. cerevisiae* in an effort to decipher the RAS signal transduction pathway.

The existence of an upstream regulator of RAS came first from the discovery of the $cdc25^{ts}$ mutants in *S. cerevisiae*, which are phenotypically similar (*9*) to mutants defective in the structural gene of adenylyl cyclase (*CDC35/CYR1*), but which can be suppressed by the RAS2(Gly^{19} → Val) mutation or by overexpressing the cyclase gene or the gene coding for the catalytic unit of cAMP-dependent kinase (*TPK1, TPK2, TPK3*) (*10*). These genetic results were interpreted to mean that CDC25 is the upstream regulator of *S. cerevisiae* RAS2, catalyzing the guanine diphosphate (GDP) to GTP exchange on RAS2. Indeed, guanyl nucleotide–dependent adenylyl cyclase activity is markedly reduced in $cdc25^{ts}$ and nullified in *cdc25*-disrupted mutants (*10, 11*). Furthermore, the rate of adenylyl cyclase activation by GTP, GPPNHP, or GTPγS is diminished in cell extracts prepared from $cdc25^{ts}$-2 cells (*12*). Overexpression of the *CDC25* gene leads to further enhancement of adenylyl cyclase activation by guanyl nucleotide in vitro, elevated intracellular cAMP in the intact cell, and shorter generation times (*12*). These findings suggest that homologs of *S. cerevisiae CDC25* that regulate other RAS proteins should exist. Indeed, the gene *ste6* in *S. pombe* is an upstream regulator of *S. pombe* RAS and has homologies to *S. cerevisiae* CDC25 and SCD25 (*13, 14*).

These findings are consistent with the biochemical experiments reported in this issue of *Science* (*15*), which show that the bacterially expressed COOH-terminal portion of SCD25 catalyzes GDP to GTP exchange on *S. cerevisiae* RAS2 protein in vitro. This finding provides the biochemical explanation for the suppression of the *cdc25* mutation by *SCD25* (*14*). However, mystery still remains. For example, these authors (*15*) could not achieve these results with the COOH-terminal portion of CDC25, which is the normal regulator of RAS2. It is possible that the SCD25 COOH-terminal fragment can catalyze an unregulated GDP to GTP exchange on RAS2 because it lacks regulatory sequences present in CDC25. These sequences may be able to respond to incoming signals that release an inhibitory structural constraint and activate the guanyl nucleotide exchange reaction. SCD25 can be viewed as a "relaxed," unregulated version of CDC25 since it is only 50% homologous to CDC25 at that region (*14*). The incoming signals to which CDC25 responds are not known, although glucose induction of cAMP requires an intact *CDC25* gene in *S. cerevisiae* (*16*).

Most exciting is the finding of Créchet and co-workers (*15*), that SCD25 COOH-terminal also catalyzes in vitro GDP to GTP exchange in bacterially expressed human H-Ras. This result suggests that the functional domain in CDC25-like proteins is conserved through evolution, as has been observed for Ras proteins. How similar SCD25 is to putative guanyl nucleotide exchange proteins for c-H-Ras and v-H-Ras (*17, 18*) remains to be elucidated. Two reports (*17, 18*) suggest the existence of an H-Ras–guanyl nucleotide exchange factor. Each group identified and partially purified a protein fraction, from bovine brain (100 kD) Triton X-100 extract (*18*) or rat brain cytosol (60 to 100 kD) (*19*), that catalyzes GDP to GTP exchange for both c-H-Ras and v-H-Ras. It is surprising that the efficiency of the GDP to GTP exchange induced by the two exchange proteins is similar for both c-H-Ras and v-H-Ras.

This GDP to GTP exchange reaction is blocked by heat inactivation (*17*) and by H-Ras–specific antibodies (*18*). It is unknown whether these two proteins are similar (or identical) to each other and whether they are members of the *CDC25/SCD25/ste6* gene product family. Because more and more small C proteins are being identified, more guanyl nucleotide exchange proteins will probably also be identified in the future.

Department of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.

REFERENCES AND NOTES

1. A. G. Gilman, *Annu. Rev. Biochem.* **56**, 615 (1987).
2. M. Barbacid, *ibid.*, p. 779.
3. F. McCormick, *Cell* **56**, 5 (1989).
4. D. Defeo-Jones *et al.*, *Nature* **306** (1983); S. Powers *et al.*, *Cell* **36**, 607 (1984); T. Toda *et al.*, *ibid.* **40**, 27 (1985).
5. G. L. Temeles *et al.*, *Nature* **313**, 700 (1985); D. Broek *et al.*, *Cell* **41**, 763 (1985).
6. S. A. Nadin-Davis, A. Nassin, D. Beach, *EMBO J.* **5**, 2963 (1987); P. M. Lund *et al.*, *Mol. Gen. Genet.* **209**, 627 (1987).
7. T. Kataoka *et al.*, *Cell* **40**, 19 (1985); S. G. Clark, J. P. McGrath, A. D. Levinson, *Mol. Cell. Biol.* **5**, 2746 (1985).
8. D. Defeo-Jones *et al.*, *Science* **228**, 179 (1985).
9. T. Camonis *et al.*, *EMBO J.* **5**, 375 (1986); V. Shilo, B. Shilo, G. Simchen, *Exp. Cell. Res.* **112**, 241 (1978).
10. D. Broek *et al.*, *Cell* **48**, 789 (1987); L. C. Robinson *et al.*, *Science* **235**, 1218 (1987); T. Toda *et al.*, *Cell* **50**, 277 (1987).
11. J. Daniel, J. M. Becker, E. Enari, A. Levitzki, *Mol. Cell. Biol.* **7**, 3857 (1987).
12. D. Engelberg, G. Simchen, A. Levitzki, *EMBO J.* **9**, 641 (1990).
13. A. Hughes, Y. Fukui, M. Yamamoto, *Nature* **344**, 355 (1990).
14. E. Boy-Marcotte, *Gene* **17**, 21 (1989).
15. J. B. Crèchet *et al.*, *Science* **248**, 866 (1990).
16. T. Munder and H. Kuntzel, *FEBS Lett.* **242**, 371 (1989); T. Munder, M. Mink, H. Kuntzel, *Mol. Gen. Genet.* **214**, 271 (1988).
17. A. Wolfman and I. G. Macara, *Science* **248**, 67 (1990).
18. M. West, H-F. Kung, T. Kamata, *FEBS Lett.* **259**, 245 (1990).