ONCOPROTEINS

GAPs in understanding Ras

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RAS proteins play a central role in neoplasia and growth control, yet we do not understand how they are controlled or what they control. The papers by Downward *et al.*[1] and Zhang and colleagues[2] on pages 719 and 754 of this issue indicate that the enigma of Ras may be yielding.

Mammalian *ras* genes were first discovered as the oncogenes of acutely transforming retroviruses[3]. Subsequently, *ras* genes with activating mutations were found in many human and rodent tumours, providing the first solid evidence that a common biochemical defect might underlie cell proliferation, or neoplasia[4]. The importance of *ras* in the scheme of growth control and neoplasia is further underscored by observations that *ras* function is required for many other oncoproteins, such as tyrosine kinases, and growth stimulants, such as the tumour-promoting phorbol esters, to have effect[5]. Moreover, *ras* genes are evolutionarily the most highly conserved oncogenes yet found, and occur in all eukaryotic organisms where they have been sought. There are great similarities between the function and regulation of *ras* in microorganisms and vertebrates, but this oncogene's main function in the yeast *Saccharomyces cerevisiae*, which is the stimulation of adenylyl cyclase, does not apparently parallel its function in vertebrates[6,7].

The *ras* genes encode a family of small, closely related proteins found at the inner surface of the plasma membrane where they can bind guanine nucleotides and slowly hydrolyse GTP to GDP[4]. Only when they are bound to GTP can the proteins fulfil their role in cell proliferation[8,9]. Rates of GTP hydrolysis by wild-type Ras protein, but not oncogenic protein, are vastly elevated *in vivo* relative to rates measured for purified Ras proteins *in vitro*[9]. These discrepancies led to the discovery of GAP, a GTPase-activating protein found ubiquitously in cells. GAP is a large protein with relative molecular mass 110,000 (110K). The carboxy-terminal 40K acts catalytically upon wild-type Ras proteins to stimulate their hydrolysis of bound GTP, but fails to stimulate GTP hydrolysis by mutant oncogenic Ras[10].

Regulation

GAP is thus an excellent candidate to be an upstream regulator of Ras protein, capable of downregulating Ras, thereby keeping it inactivated in the GDP-bound state. Oncogenic Ras proteins would escape this regulation. The reports in this issue[1,2] strongly support this notion. First, Zhang *et al.*[2] report that transfection of the GAP gene inhibits morphological transformation in NIH 3T3 indicator cells by the normal H-*ras* gene, but not by the activated mutant v-*ras* gene. These results provide the first direct evidence that GAP can inhibit Ras function in mammalian cells. It was already known that GAP has sequence similarities to the *IRA* gene products of *S. cerevisiae*, which downregulate yeast *ras* genes[11]. Mammalian GAP, if expressed in yeast, can complement the loss of the *IRA-1* gene, and can inhibit the wild-type mammalian H-*ras* but not the activated oncogenic form when coexpressed in yeast[12].

Downward *et al.*[1] provide a deeper insight into the factors that regulate Ras function. They report for the first time a physiologically relevant event that leads to activation of Ras: stimulation of the immune system's T-cell receptor. The amount of Ras in the GTP-bound state in T cells rapidly rises tenfold when cells are stimulated with PHA or a monoclonal antibody directed to the T-cell receptor. Stimulation of the T-cell receptor is thought to activate phospholipase C, which can generate at least two intracellular messengers: diacylglycerol, which activates protein kinase C, and inositol phosphates, which can mobilize intracellular calcium. Downward *et al.* demonstrate that the activation of protein kinase C by the tumour-promoting phorbol esters increases even more rapidly the proportion of Ras bound to GTP in T cells.

Similar but lesser effects were observed in other cell types. In principle, an increase in the ratio of Ras bound with GTP to Ras bound with GDP could result either from an increase in the rate of nucleotide exchange on Ras or from an inhibition of GTP hydrolysis, owing, for example, to an inhibition of GAP activity. In fact, Downward *et al.* observed no alteration in the rate of nucleotide exchange on Ras upon treatment with phorbol ester, but they found a rapid decrease in GAP activity in treated cells, suggesting that a GAP-like protein, lying in the signal transduction pathways of T-cell stimulation and protein kinase C activation, regulates Ras.

The question of the placement of GAP in the Ras signal transduction pathway is not so easily resolved. Mutant forms of Ras which fail to have effector function also fail to interact properly with GAP, leading to the hypothesis that GAP may indeed be a target of Ras action[13,14]. This remained an intriguing but weak hypothesis until recently, when Yatani *et al.*[15] reported that GAP and Ras seem to cooperate in the inhibition of coupling between muscarinic receptors and atrial potassium channels. Each protein, purified, can inhibit this coupling alone, as can be demonstrated by an *in vitro* patch-clamp preparation, and antibodies to either component can block the activity of the other. Yatani *et al.* speculate that a Ras·GAP complex may alter membrane phospholipid metabolism.

Effector function

Do the results of Yatani *et al.* conflict with those of Zhang *et al.*? Not necessarily. The latter are compatible with two possibilities. First, endogenous levels of GAP in cells may not be limiting for Ras effector function. High levels of exogenous GAP might decrease Ras function if the amount of GAP-bound Ras exceeds the levels of a stoichiometric target for GAP. Second, Ras might have more than one effector function in cells. It is clear from the comparative studies that Ras has had at least two effector functions in the course of evolution. Moreover, evidence has accumulated that Ras has more than one primary effector function in *S. cerevisiae*[16]. These observations serve as a warning that Ras effector functions in mammalian cells are likely to be complex.

Hope for the resolution of the function of GAP has flared anew with the riveting report by Xu *et al.*[17] that *NF-1*, the locus involved in hereditary neurofibromatosis, encodes a protein that is similar to GAP and to the yeast *IRA-1* gene product[18] (see also Ponder's News and Views article on page 703 of this issue). The similarity between the *NF-1* gene product and GAP is restricted to the domain that catalyses accelerated GTP hydrolysis by Ras. But, the similarity between NF-1 and IRA-1 extends well beyond this region, and NF-1 is consequently closer to IRA-1 than it is to mammalian GAP. This result suggests that there may be a family of GAP-like molecules which may interact with Ras.

Patients with a single copy of the NF-1 disease locus are prone to sporadic benign growths of neuroectodermal origin, and hence, if hereditary neurofibromatosis

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follows the pattern of hereditary retinoblastoma, the NF-1 gene may be an anti-oncogene. If its product holds in check the activity of Ras, or a Ras-like protein, the sporadic loss of the good allele in the cells of NF-1 patients would then lead to aberrations in the growth control of these cells due to the constitutive activation of Ras. In this interpretation, the *NF-1* gene product acts as an upstream negative regulator of Ras.

There is, however, a contradictory interpretation. In some cells of neuroectodermal origin, Ras stimulates differentiation and blocks proliferation[19,20]. If *NF-1* encodes the target for Ras action in neuroectodermal cells, the loss of this product might also lead to uncontrolled cellular proliferation. Thus, for now, the striking news about *NF-1* is perfectly ambiguous. □

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SILICON CLUSTERS

All surface and no activity

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ELEMENTARY considerations of solid geometry show that as the radius of a sphere is reduced, the ratio of surface area to volume increases. If this picture is extrapolated to an atomic scale it is easy to appreciate that for a small collection of atoms — a cluster — such as Si_{24}, the majority of atoms reside on the surface. Given such circumstances it is, therefore, surprising to note that in a recent study of silicon clusters, Jarrold *et al.*[1] observed the clusters to be far less reactive for molecular oxygen than the surface of bulk silicon.

The technical importance of silicon in the fabrication of electronic devices has meant that processes like the oxidation of well characterized surfaces have been thoroughly investigated. With the trend towards microelectronics and the possibility of constructing nanometre-scale (10^{-9} m) devices[2], there is clearly a need to identify how the chemical and physical properties of a bulk metal or semiconductor change when reduced to atomic proportions; the study of clusters could fulfil that requirement.

Clusters from refractory materials, such as silicon, are best prepared using laser vaporization; an intense pulse of laser radiation strikes a metal surface, vaporizing material which is then caught in the flow of a buffer gas such as helium. The gas cools the vapour, and promotes nucleation, resulting in a selection of clusters of various sizes. As will be seen, the efficiency of the cooling process could have a key role in determining the reactivity of the clusters.

A complicating factor in studying reactivity is that the comparisons are made between ionic clusters and neutral surfaces of bulk silicon. Experimental convenience dictates that ionic clusters are used; the electric charge means that clusters can be mass-selected and guided to the reaction zone. In the event, delocalization of the charge diminishes the magnitude of any electrostatic interactions between a cluster and a reactant molecule, and calculations suggest that there is very little difference between the structures of large neutral and ionic silicon clusters.

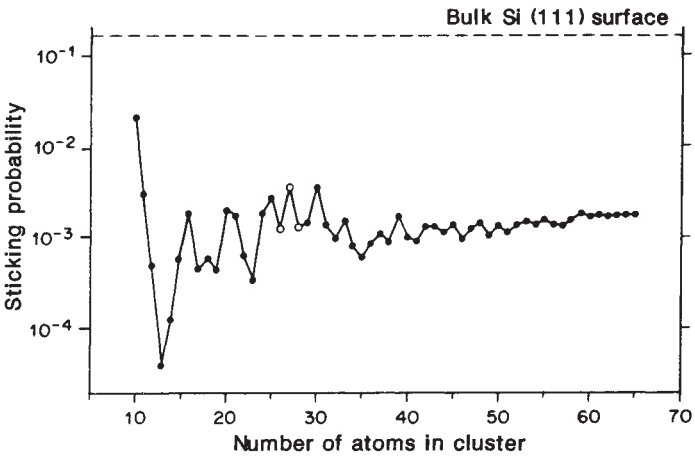

FIG. 1 Estimated sticking probability for the collision between an Si_n^+ cluster and an oxygen molecule as a function of cluster size. Shown as a dashed line is the result for the bulk Si(111) surface. (From ref. 1.)

Similar experimental considerations apply to the reaction products: charged products are readily identified using mass spectrometry, whereas the nature of neutral fragments can often be inferred from the thermochemistry. Thus, in the case of a reaction between a silicon cluster ion and an oxygen molecule, the dominant steps are[1]:

$$Si_n^+ + O_2 \rightarrow Si_{n-2}^+ + 2SiO \text{ or } Si_2O_2 \quad (\text{for } n < 30) \qquad (1)$$

$$\rightarrow Si_nO_2^+ \quad (\text{for } n > 30) \qquad (2)$$

The absence of detailed information on any neutral reaction products, together with the observation of step (2), supports the approach taken by Jarrold *et al.*[1] in interpreting the experimental data in terms of sticking probabilities. On this basis, a direct comparison with bulk silicon[3] suggests that the probability of O_2 sticking on a cluster is lower by a factor of 100. Although the reactivity of smaller clusters with oxygen varies significantly with size (Fig. 1), beyond Si_{25}^+ the sticking probability is almost constant as a function of size.

Jarrold[4] recently compiled a list of other molecules, such as methanol and ammonia, whose reactivity varies markedly with the size of the target silicon clusters. Possibly the most dramatic examples have come from the work of Smalley and co-workers[5], who found that the reactivities of the clusters Si_{39}^+ and Si_{45}^+ with ammonia and ethene are several orders of magnitude lower than those of clusters one atom smaller or larger.

In a novel extension to those experiments, Smalley's group has shown[6] that the reactivity of a silicon cluster ion can be modified through laser-induced annealing. In particular, annealing renders Si_{39}^+ and Si_{45}^+ virtually inert with respect to ethene and ammonia. But note that Jarrold *et al.*[1] did not see a similar discrimination on the part of these large clusters against oxygen. Such marked differences in behaviour between what are in essence identical experiments suggests that it may be necessary to consider in detail the way the silicon clusters are produced.

Laser vaporization leads to the generation of a plasma consisting of atoms, ions and electrons. The gas pulse which entrains the vaporized plume quenches the plasma and initiates nucleation. But because the binding energy between silicon atoms is quite high (the heat of atomization is about 4.6 electron volts), it is possible to produce 'stable' clusters with quite high internal temperatures, and only through further collisions and/or evaporation will they become annealed. Depending on the exact conditions, three types of silicon cluster could emerge from the vaporization process: hot and possibly liquid-like; annealed, but to a metastable structure; or annealed to the ground-state structure.

Clusters from the first option might behave in a manner analogous to a hot surface, having low sticking probabilities owing to the rapid desorption of molecules. Beck and Andrews[7] recently provided evidence of a laser-induced phase transition between solid-like and liquid-like forms of silicon clusters. Bearing in mind that the melting point of a cluster could be at least a factor of two lower than that of the bulk material[8], estimates of the (photon) energy required to 'melt' a cluster may need to be re-evaluated. The possibility of the annealed options suggests the existence of structural isomers. These