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Structure and Function of *ras* and *Blym* Oncogenes

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A. Introduction

The biologic activity of tumor DNA, detected by transfection of NIH 3T3 mouse cells, has led to the identification of transforming genes which are activated in a variety of human, rodent, and avian neoplasms (see [5] for recent review). The transforming genes of some neoplasms are cellular homologs of the *ras* genes of Harvey and Kirsten sarcoma viruses. Other neoplasm transforming genes, such as *Blym-1*, are unrelated to *ras* or to other retroviral transforming genes. In this article, I will discuss recent work related to functional analysis of the *ras* and *Blym-1* gene products.

B. *ras* Genes

Three different members of the *ras* gene family have been identified as biologically active transforming genes in neoplasm DNA: ras^H, ras^K, and ras^N [8, 15, 16, 18, 22]. These genes have been detected in many different types of neoplasms, including carcinomas, sarcomas, melanomas, neuroblastomas, lymphomas, and leukemias of myeloid and lymphoid origin. Thus, it appears that *ras* genes can contribute to the development of neoplasms arising from multiple types of differentiated cells. This is consistent with the fact that *ras* genes are expressed in all normal vertebrate cells which have been examined. In addition, yeast contains functional *ras* genes, suggesting that these genes play a fundamental role in cell proliferation which is highly conserved in evolution. However, *ras* genes are found as active transforming genes in only a small fraction (approximately 10%–20%) of individual neoplasms. Thus, although *ras* activation can occur in many different types of tumors, it is apparently not a necessary event for development of any particular type of neoplasm. In addition, recent data suggest that *ras* activation may be a late event in tumor progression. For example, Albino et al. [1] have reported detection of an activated ras^N gene in only one in five metastases of an individual melanoma patient. This finding suggests the possibility that *ras* activation may, when it occurs, impart a selective advantage to a clone of neoplastic cells, but is not essential for formation of a primary neoplasm or even its metastatic derivatives.

The *ras* genes all encode proteins of approximately 21 000 daltons which are designated p21. Experimental manipulations of the normal human ras^H gene have shown that overexpression of the normal gene product is sufficient to induce cell transformation [4]. However, activation of *ras* genes in human tumors is commonly a consequence of structural, rather than regulatory, mutations [2, 3, 7, 17, 21, 23–25, 27]. The mutations in tumors which have been analyzed to date alter either codon 12 or codon 61. At either of these positions, substitution of multiple different amino acids is sufficient to endow p21 with transforming activity. In addition, most activat-

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ing mutations appear to induce conformational alterations in p21 which are detectable by abnormal electophoretic mobilities [7, 23, 27]. Taken together, these observations suggest that substitution of a variety of abnormal amino acids at these critical loci may inactivate a regulatory domain of p21, thus resulting in abnormal p21 function in vivo.

Studies of viral *ras* proteins have indicated that they are localized at the inner face of the plasma membrane [12, 26] and modified by acylation [20]. The only established biochemical activity common to all viral *ras* transforming proteins is guanine nucleotide binding [12, 19].

To attempt to elucidate the biochemical basis for the transforming activity of mutant p21 in human tumors, we have compared the biochemical properties of p21 encoded by normal and activated human *ras* genes. These experiments indicated that both normal and transforming human p21 were localized at the plasma membrane and were modified to similar extents by posttranslational acylation [11]. Neither normal nor activated p21 were glycosylated or phosphorylated [7, 11]. Thus, the subcellular localization and posttranslational processing of human p21 were not altered by *ras* gene activation.

Since guanine nucleotide binding represented the only known biochemical activity of p21, we investigated the possibility that the affinity or specificity of p21 for nucleotides was altered as a consequence of mutational activation. However, the GTP binding affinities of both normal and activated human p21 were indistinguishable ($K_D = 1{-}2 \times 10^{-8}$ *M*) and both the normal and activated proteins were specific for GTP and GDP binding [11]. Thus, mutational activation of p21 does not directly affect its intrinsic nucleotide binding properties.

In order to investigate the physiologic function of *ras* proteins, we have attempted to identify other cellular proteins with which p21 might interact [10]. Immunoprecipitation of extracts of human carcinoma cell lines with anti-p21 monoclonal antibodies revealed the coprecipitation of a second protein of approximately 90 000 daltons. This coprecipitated protein was identified as the transferrin receptor by three criteria: (a) comigration in both reducing and nonreducing gels; (b) immunologic reactivity with monoclonal antibody raised against transferrin receptor; and (c) identity of partial proteolysis maps of the 90 000 daltons coprecipitated protein and transferrin receptor. Coprecipitation of transferrin receptor was detected with three different *ras* monoclonal antibodies and was dependent on the presence of *ras* proteins in cell extracts, indicating that p21 and transferrin receptor form a molecular complex. This complex was dissociated by addition of transferrin to cell extracts, suggesting that transferrin binding induced a conformational change in the receptor which led to the dissociation of *ras* proteins.

Transferrin is an iron binding protein which is required for the growth of most cells in culture. Expression of transferrin receptor is closely correlated with cell proliferation. Furthermore, monoclonal antibodies against transferrin receptor inhibit cell growth, in some cases even if iron is supplied in an alternate form. Transferrin and its receptor thus appear to play a fundamental role in the growth of many differentiated cell types. The findings of interaction between *ras* proteins and transferrin receptor therefore suggest that p21 may function in conjunction with this cell surface receptor in regulation of cell growth, perhaps by transducing growth signals mediated by transferrin binding. It is possible that the role of p21 in this respect is analogous to other membrane guanine nucleotide binding proteins, such as the adenyl cyclase G proteins and transducin [13].

C. *Blym* Genes

In contrast to *ras*, the *Blym*-1 gene is representative of several transforming genes which are activated highly reproducibly in neoplasms of specific cell types. Thus, *Blym*-1 has been detected as an active transforming sequence in all chicken B cell lymphomas [6] and all human Burkitt's lymphomas [9] which have been examined. It thus appears to play a highly reproduc-

ible role in the development of similar B cell neoplasms in both chickens and humans.

The *Blym*-1 transforming gene activated in chicken B cell lymphomas was isolated as a molecular clone by sib-selection [14]. The cloned chicken *Blym*-1 gene was unusually small (only about 600 nucleotides) and its nucleotide sequence indicated that it encoded a small protein of 65 amino acids [14]. Comparison of the predicted chicken *Blym*-1 amino acid sequence with sequences of known cellular proteins revealed partial homology (36%) between the chicken *Blym*-1 protein and the NH, terminal region of transferrin family proteins [14]. This homology was concentrated in regions which were conserved between different members of the transferrin family, suggesting a common ancestry for chicken *Blym*-1 and a region of the transferrins, as well as stimulating the speculation that this homology might also suggest a functional relationship.

Blot hybridization analysis indicated that the chicken *Blym*-1 gene was a member of a small family of related genes which were present in human as well as chicken DNA. We therefore investigated the possibility that the transforming gene detected by transfection of Burkitt's lymphoma DNA might be a member of the human gene family defined by homology to chicken *Blym*-1. A genomic library of DNA from a Burkitt's lymphoma was screened using chicken *Blym*-1 probe and a biologically active human transforming gene, designated human *Blym*-1, was isolated [9]. This human homolog of chicken *Blym*-1 was found to represent the transforming gene detected by transfection of all six Burkitt's lymphoma DNA samples studied.

Restriction mapping and nucleotide sequencing indicate that human *Blym*-1, like chicken *Blym*-1, is quite small (approximately 700 nucleotides) (Diamond et al., manuscript submitted [9]). Also like chicken *Blym*-1, the sequence of human *Blym*-1 predicts a small protein (58 amino acids) which consists of two exons and is rich in lysine and arginine. Alignment of the human and chicken *Blym*-1 amino acid sequences indicates 33% amino acid identities. The human and chicken *Blym*-1 proteins are therefore clearly related ($P < 0.005$), but significant divergence between the two sequences has occurred. This divergence suggests the possibility that the chicken and human genes may represent relatively distant members of the *Blym* family.

In spite of the divergence between the chicken and human *Blym*-1 genes, the human *Blym*-1 sequence also displays significant homology (20%) to the NH_2 terminal region of transferrins. Significantly, amino acids which are conserved between the chicken and human *Blym*-1 genes also tend to be conserved between different members of the transferrin family. It is unlikely that such divergent sequences as chicken and human *Blym*-1 have maintained homology to transferrin by chance. Rather, the conservation of transferrin homology in these *Blym* transforming genes suggests that this homology reflects some functional property of the *Blym* transforming proteins. In view of the molecular interaction between *ras* proteins and transferrin receptor, these findings suggest the hypothesis that the *Blym* transforming genes may also affect cell proliferation via a pathway related to transferrin and its surface receptor. Further understanding of the function of the *Blym* gene products will require direct biochemical analysis of these proteins.

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