Molecular Events in Cells Transformed by Rous Sarcoma Virus

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ABSTRACT The Rous sarcoma virus (RSV) transforming gene product has been identified and characterized as a phosphoprotein with a molecular weight of 60,000, denoted pp60src. Partially purified pp60src displays a closely associated phosphotransferase activity with the unusual specificity of phosphorylating tyrosine residues in a variety of proteins. That the enzymatic activity observed is actually encoded by the RSV-transforming gene is indicated by the comparison of the pp60src-protein kinase isolated from cells transformed by a wild-type RSV or by a RSV temperature-sensitive transformation mutant; these experiments revealed that the latter enzyme had a half-life of 3 min at 41°C, whereas that of the wild-type enzyme was 20 min. Evidence is now beginning to accumulate showing that viral pp60src expresses its protein kinase activity in transformed cells as well as in vitro because at least one cellular protein has been identified as a substrate for this activity of pp60src.

Although the protein kinase activity associated with pp60src is itself cyclic AMP (cAMP) independent, the molecule contains at least one serine residue that is directly phosphorylated by the cellular cAMP-dependent protein kinase, thus suggesting that the viral transforming gene product may be regulated indirectly by the level of cAMP. The significance of this latter observation must be regarded from the point of view that the RSV src gene is apparently derived from a normal cellular gene that seemingly expresses in normal uninfected cells a phosphoprotein structurally and functionally closely related to pp60src. This cellular protein, found in all vertebrate species tested, also is a substrate for a cAMP-dependent protein kinase of normal cells, and, therefore, may have evolved to function in a regulatory circuit involving cAMP.

Many of the phenotypic changes characteristic of cell transformation have been reviewed by Hanafusa (1). They include altered cellular morphology and the ability of transformed cells, but not normal cells, to grow in soft agar. At the morphological level, scanning electron microscopy reveals increased cell surface activity (7) and modification of the cytoskeletal components in transformed cells (8). Various other biochemical events also occur, such as increased hexose transport, increased secretion of plasminogen activator, and loss from transformed cells of the surface glycoprotein fibronectin (1, 9, 10).

To follow the sequence of events in RSV-infected cells that lead to cell transformation, identification of the product of the src gene was required. Hopefully, extensive characterization of this product and of its functions will lead to a detailed description of the molecular changes in RSV-transformed cells.
Identification of the src Gene Product

The genetic evidence cited above had indicated by 1972 that the src gene product was likely to be a protein, however, it has only recently been identified. We used two approaches in our search for the src gene product. One utilized serum from mammals bearing virus-induced tumors for immunoprecipitation of radiolabeled proteins from transformed cells; a technique that had proved successful in the identification of non-structural virus-encoded polypeptides of DNA tumor viruses (11, 12). The other was cell-free translation of the subgenomic 3'-third of the RSV genome, the region of the genome that had previously been shown to contain the src gene (13). These techniques led to the identification of a transformation-specific antigen in all RSV-transformed cells examined (14, 15) and to the identification of a polypeptide among the products of cell-free translation that was synthesized only when src-containing RNA was translated (16). The transformation-specific antigen found by immunoprecipitation of extracts of transformed cells and the src-specific cell-free translation product both had the same molecular weight, 60,000, and similar methionine-containing tryptic peptides (17). Similar results have been reported from other laboratories as well (18, 19). The transformation-specific polypeptide immunoprecipitated from transformed cells proved to be a phosphoprotein (15, 20) and was, thus, designated pp60"src" to denote its size, secondary modification, and genetic origin. These results are summarized, in part, by the data shown in Fig. 1.

Characterization of the Phosphorylation Sites in pp60"src"

When pp60"src" was purified by immunoprecipitation from transformed cells that had been radiolabeled in culture with [32P]orthophosphate and then digested with the proteolytic enzyme V8 from *Staphylococcus aureus*, two large fragments were generated, both of which contained phosphorylous radiolabel. Experiments showed that these two fragments corresponded to the amino and carboxy terminal portions of the molecule, and, thus, we reported that pp60"src" had two major sites of phosphorylation (21). Phosphoamino acid analysis of the amino-terminal fragment revealed that it contained a phosphoserine residue, as judged by electrophoresis at pH 1.9, whereas the same analysis applied to the carboxy-terminal fragment showed that the phosphorylated residue comigrated with phosphothreonine. More recently, it was demonstrated that the nonserine phosphoamino acid is tyrosine not threonine (22), and we have subsequently confirmed these results. Two-dimensional fractionation is required to separate phospho-lysine from phosphothreonine because they comigrate at pH 1.9.

In our original studies (21), we demonstrated that the serine residue is phosphorylated by a cyclic AMP (cAMP)-stimulated protein kinase activity in cell-free extracts. This is of considerable interest because established with other substrates of the cAMP-dependent protein kinase indicates that their function is influenced by their phosphorylation state (23), and the same may prove true for pp60"src". Furthermore, our recent unpublished results show that partially purified pp60"src" is a direct substrate of the catalytic subunit of the cAMP-dependent protein kinase in vitro. Thus, an enzyme potentially able to regulate the activity of the transforming gene product of RSV is present in both normal and transformed cells.

In contrast to the serine residue, our cell-free phosphorylation studies of the tyrosine residue showed that it is phosphorylated by a cAMP-independent protein kinase activity (21). Furthermore, this same series of experiments revealed that the phosphotyrosine residue on the carboxy-terminal V8 fragment is not phosphorylated in pp60"src" encoded by ts transformation mutants of RSV when is mutant-transformed cells are grown at the nonpermissive temperature (normal morphology), whereas phosphorylation is similar to that of pp60"src" encoded by wild-type virus when is mutant-transformed cells are grown and radiolabeled at the permissive temperature (transformed morphology). These data are summarized in Fig. 2.

Our unpublished results indicate that the turnover of the phosphate on each residue is very rapid. In the case of the tyrosine site, the half-life of the phosphate is <1 h. Therefore, pp60"src" may exist in several forms with regard to its state of phosphorylation. Thus far, we have not measured the quantity of each form because we have been unable to resolve discrete species of the molecule based on phosphate content.

A Function Associated with pp60"src"

Early results from our laboratory indicated that most pp60"src" is located in the cytoplasm of transformed cells (24) and is present at relatively low levels. These observations, together with the variety of alterations produced by the expression of pp60"src", suggested that it may have an enzymatic function resulting in the modification of several substrates. Because
phosphorylation-dephosphorylation reactions are apparently involved in the regulation of many cellular processes (25–27), we searched for a protein kinase activity associated with pp60<sup>src</sup>. In our initial experiments (28), when we tested specific immunoprecipitates that contained pp60<sup>src</sup> for protein kinase activity we found that no substrates added exogenously, such as histone or casein, were phosphorylated, whether or not pp60<sup>src</sup> was present. However, immunoprecipitates that contained pp60<sup>src</sup> did catalyze the transfer of radiolabel from [γ-<sup>32</sup>P]ATP to the heavy chain of IgG. Immunoprecipitates that lacked pp60<sup>src</sup> showed no activity in this assay. In addition, we found a similar activity associated with pp60<sup>src</sup> generated by cell-free translation of subgenomic viral RNA that contained the src gene (29). Others have also reported similar results from their studies on pp60<sup>src</sup> (20, 30). Thus, it appeared that the presence of pp60<sup>src</sup> was an obligatory requirement for the IgG kinase activity. This unexpected observation has proven to be a useful assay for additional studies on pp60<sup>src</sup>. This assay is diagrammed in Fig. 3. These data indicated that phosphotransferase activity was not inhibited in the immune complex and, in this regard, it is interesting that other results from this laboratory have shown that some antisera from RSV tumor-bearing mammals could immunoprecipitate viral DNA polymerase without inhibiting its enzymatic activity.

Fractionation by conventional biochemical procedures using ion-exchange chromatography together with the assay of fractions for both biosynthetically radiolabeled pp60<sup>src</sup> and IgG kinase activity revealed that pp60<sup>src</sup> and IgG kinase activity coeluted under all conditions examined to date (31, 32). Ion-exchange and immunofinity chromatography yielded preparations of pp60<sup>src</sup> sufficiently pure to permit the characterization of the protein kinase activity during conventional soluble reactions. When substrates routinely used in such assays were surveyed, only casein showed detectable phosphate-acceptor activity. Moreover, as shown in Fig. 4, this casein kinase activity was inhibited by anti-pp60<sup>src</sup> IgG, but not normal IgG, added before the reaction. Our interpretation of this result is that pp60<sup>src</sup>, when sequenced by antibody, is unable to interact with the casein substrate properly and no phosphorylation occurs. Although no information was presented regarding the quantity or presence of pp60<sup>src</sup>, others using the criterion of IgG kinase activity arrived at a similar conclusion (33). It is also evident in Fig. 4 that the phosphotransferase reaction carried out on pp60<sup>src</sup> with no additions resulted in an apparent "autophosphorylation." In this case, radiolabel is incorporated into the same tyrosine-containing phosphopeptide as is found after biosynthetic labeling (31). This result has been noted in Fig. 2.

Despite the accumulation of experimental data, biochemical evidence alone remained insufficient to establish the origin of the protein kinase because the possibility remained that another polypeptide undetectable by our techniques was the actual kinase acting in conjunction with pp60<sup>src</sup>. Therefore, we also used mutants of RSV that were in their transforming function on the assumption that the function of the transforming protein encoded by these mutants should be more thermolabile than that of the wild-type parental virus. Protein kinase activity associated with pp60<sup>src</sup> was partially purified from chicken cells transformed by NY68, a ts transformation mutant (5), and compared with that isolated from cells transformed by its wild-type parent. These assays showed that the protein kinase activity of the mutant in both the soluble reaction and in the immune complex IgG phosphorylation was at least sevenfold more thermolabile than that of wild-type virus, demonstrating more directly that the RSV src gene product is likely to have a protein kinase function (32). Using ts mutants but different experimental approaches, others have reached similar conclusions (20, 34). It must be emphasized, however, that this result does not eliminate the possibility that pp60<sup>src</sup> may have other functions important for its role in transformation.

During their studies which led to the identification of phos-
mediated via phosphorylation. To establish a role for phospho-
infected cell as a protein kinase or that transformation is
pp60' in vitro does not establish that pp60'- acts in the
complex assay occurred at tyrosine. Work in this laboratory
with pp60` has an unusual specificity because many other
specific kinases are present.

Identification of a Substrate for pp60<sup>src</sup>

The demonstration of a protein kinase activity tightly asso-
ciated with pp60<sup>src</sup> has obvious implications with regard to
cellular transformation because protein phosphorylation/de-
phosphorylation reactions are involved in various cellular reg-
ulation mechanisms (23, 25-27). For example, as was originally
suggested (28), one might assume that pp60<sup>src</sup> activity causes a
quantitative or qualitative change in the phosphorylation pat-
terns in the infected cell resulting in transformation that could
be readily reversible by phosphatases. This could account for
the observations made with is transformation mutants (37),
which show that at least normal morphology can be restored
with pp60<sup>src</sup>-activity in the infected cell as a protein kinase or that transformation is
mediated via phosphorylation. To establish a role for phospho-
rylation-dephosphorylation in RSV-induced transformation, a
cellular protein substrate(s) had to be identified that was
phosphorylated directly by pp60<sup>src</sup> activity in the cell. In ad-
dition, some functional modification associated with this phos-
phorylation must be characterized and shown to lead to at least
one phenotypic change in the cell. Because there are so many
changes in response to the src gene product, more than one
substrate might be anticipated.

A number of experiments performed by several different
groups (37, 38) have led to the frequent suggestion that the
cellular cytoskeleton is likely to include one target of the src
gene product essential for the transformation process. With
the availability of anti-pp60<sup>src</sup> serum, as stated above, it quickly
became clear that pp60<sup>src</sup> was located primarily in the cyto-
plasm of transformed cells; however, the issue of a subcyto-
plasmic localization appears open to debate. It has been re-
ported that the location of pp60<sup>src</sup> is primarily perinuclear (39),
whereas others have shown it to be highly concentrated im-
mediately under the plasma membrane (40). Some results
suggest that it is rather tightly complexed to the cytoskeleton
after detergent extraction of whole cells (41), whereas on the
other hand, it is also found in cellular membrane preparations
(42). More recently, Rohrschneider (43) has reported that
pp60<sup>src</sup> is located in adhesion plaques of transformed cells,
certainly a pivotal location when considered with regard to the
morphological changes induced by RSV. There may be no
inconsistency in these results because the presence of pp60<sup>src</sup>
at several sites may be essential for the full spectrum of changes
observed in transformed cells. Whether or not pp60<sup>src</sup> is ulti-
imately assigned to a precise and correct subcellular location(s),
investigators are not, unfortunately, relieved of finding and
suitably characterizing a substrate(s) among the large number
of cellular polypeptides that remain with the subcellular frac-
tion under study. Nor does localization at a particular site
imply that pp60<sup>src</sup> located elsewhere is silent in the transfor-
mation process.

Although attention is now directed at the cytoplasm and
particularly at cytoskeletal or membrane components as pri-
mary targets for pp60<sup>src</sup> activity, the results which reveal that
changes in certain mRNA levels also occur in RSV-trans-
formed cells should be considered. Transcriptional activation
of cellular genes in RSV-transformed cells has been found, as
judged by the presence of new mRNA transcripts and increased
sensitivity of certain genes to deoxyribonuclease I, an assay for
transcriptionally active chromatin (44, 45). Other studies, show-
ing greatly decreased levels of collagen mRNAs, also imply
alterations in transcriptional controls although other explana-
tions are available (46). These changes may result from pp60<sup>src</sup>
action on primary targets in the cytoplasm that only indirectly
alter transcription but, nevertheless, may have important con-
sequences with regard to sarcoma formation.

Some progress has been made in the identification of sub-
strates phosphorylated by pp60<sup>src</sup> without regard to subcellular
location. Total phosphoproteins from normal and transformed
cells have been solubilized and analyzed by the techniques
described by O'Farrell et al. (47). This procedure involves
fractionation of proteins based on isoelectric point and molecu-
lar weight, permitting the identification of a newly phosphy-
rlated protein by a shift in its isoelectric point. The transfor-
mation-specific phosphorylation of a protein has been detected
by application of this procedure (31, 48). This protein has a
molecular weight of ~34,000 and a pI of ~7.5. Radke and
Martin (48) reported that this protein was phosphorylated

within 20 min after shifting is transformation mutant-infected cells from the nonpermissive to permissive temperature. Thus, the phosphorylation of this protein is one of the earliest biochemical markers of transformation. We have recently purified the unphosphorylated form of this protein from normal cells and have shown that partially purified pp60<sup>src</sup> will phosphorylate it in vitro (Fig. 5). Comparative phosphopeptide fingerprints reveal that the site phosphorylated in vitro occurs at a tyrosine residue in a tryptic peptide similar or identical to the major phosphopeptide found in the 34,000-dalton phosphoprotein isolated from transformed cells (49). Taken together, these results suggest that this normal cell protein is phosphorylated directly as a consequence of pp60<sup>src</sup> activity. These data do not show, however, that this particular phosphorylation is crucial for transformation. Extension of these studies will require functional characterization of the phosphorylated and unphosphorylated forms of this protein. Nevertheless, these data do show more directly that the protein kinase activity associated with pp60<sup>src</sup> in vitro is likely to be a biologically significant function of the molecule in the transformed cell.

**Normal Cellular Homologue of the RSV src Protein**

It is relevant to this discussion that both normal avian and mammalian cells contain a phosphoprotein antigenically, structurally, and perhaps functionally related to pp60<sup>src</sup>. Molecular hybridization experiments with radioactive DNA specific for the RSV src gene have shown that normal uninfected vertebrate cells contain highly conserved nucleotide sequences, denoted src, that are related to the viral transforming gene and that are present in both DNA and RNA (50–53). Furthermore, src-containing RNA has been found to be associated with polyribosomes, suggesting that this RNA may be translated into a protein. The close relationship in nucleotide sequence between normal cell src and viral src implies that the putative src protein may be Similar in structure and function to the viral pp60<sup>src</sup>. We, therefore, attempted to identify a src-related protein in normal, uninfected cells. We (54, 55) and others (56–58) have found a 60,000-dalton phosphoprotein in normal avian and mammalian cells that is antigenically related to the viral src protein (Fig. 6). This protein was identified by immunoprecipitation of radiolabeled cell extracts with certain cross-reacting sera derived from RSV tumor-bearing mammals. In addition to being antigenically related, all three classes of proteins (viral, avian cell, and mammalian cell) appear to be structurally very similar but not identical.

Comparative studies of the sites of phosphorylation on these polypeptides have shown that the normal cell src-related protein also contains both phosphoserine and phosphotyrosine, with the former being located on the V8 amino-terminal peptide and the latter on the V8 carboxy-terminal fragment (59). Furthermore, the serine residue of the normal cell src-related protein is also a direct substrate of purified cAMP-dependent protein kinase and is located in a tryptic peptide similar, if not identical, to that from viral pp60<sup>src</sup> (35, 59). Thus, the function of this protein, like that of viral pp60<sup>src</sup>, is likely to be regulated indirectly by the level of cAMP in cells.

Because the normal cell 60,000-dalton protein is antigenically and structurally so similar to the viral src protein, it appears to fit the description expected of the protein product of the cellular src sequences, and we have, therefore, designated it pp60<sup>src</sup>. The close relationship among the normal endogenous src polypeptides and the RSV src gene product suggests that these apparently highly conserved proteins may have a similar function. Along these lines, we (59) and others (56) have identified a protein kinase activity associated with pp60<sup>src</sup> in a manner similar to that described for viral pp60<sup>src</sup> (28). The similarity of the results obtained with both viral pp60<sup>src</sup> and normal cell pp60<sup>src</sup> in the immune complex protein kinase assay suggests that the two proteins may have similar functions resulting in the phosphorylation of tyrosine residues in polypeptides.

**FIGURE 5** In vitro phosphorylation of the 34,000-dalton normal cell protein by pp60<sup>src</sup>. A preparation of the 34,000-dalton protein purified from normal chicken embryo fibroblasts was incubated in the protein kinase reaction mixture and the products of the reaction were analyzed by polyacrylamide gel electrophoresis and autoradiography. Left panel, a Coomassie Blue-stained SDS-polyacrylamide gel after electrophoresis of the partially purified preparation. Autoradiogram of SDS-polyacrylamide gel analysis of protein kinase reaction products: track 1 34,000-dalton protein preparation alone, 2 34,000-dalton protein preparation with partially purified pp60<sup>src</sup>, 3 with pp60<sup>src</sup> and normal rabbit IgG, 4 with pp60<sup>src</sup> and immune IgG from tumor-bearing rabbits, and 5 pp60<sup>src</sup> preparation alone.

**FIGURE 6** Identification of the normal cell homologue of pp60<sup>src</sup>. Normal chicken embryo fibroblasts were labeled in culture with either [<sup>35</sup>S]methionine ([<sup>35</sup>S]-Met) or [<sup>32</sup>P]orthophosphate ([<sup>32</sup>P]). Cell extracts were prepared and immunoprecipitated with normal rabbit serum (n) or cross-reacting tumor-bearing rabbit serum (i). The immunoprecipitated materials were analyzed by SDS-polyacrylamide gel electrophoresis and fluorography.
Supporting this possibility are the results obtained by Hanafusa and co-workers (60) involving the recovery of transforming viruses from tumors arising in chickens infected with transformation-defective src-gene deletion mutants of RSV. It appears that these recovered transforming viruses are generated by recombination between viral and cellular (presumably src) RNA or DNA sequences to create a functional src gene (61). These recovered viruses have obtained at least 75% of their transforming gene from cellular sequences and they produce a protein closely related to pp60csrc during transformation of cells in culture. Moreover, the transforming gene product identified shows an association with a protein kinase activity, as judged by the immune complex assay (62).

Several explanations may be considered as to why pp60src expression is compatible with normal cellular proliferation. First, as mentioned above, it is possible that the src and src-related polypeptides have functions other than protein phosphorylation, whereby the roles of viral and cellular pp60s differ. Alternatively, both the viral src protein and its normal cell homologue may function as protein kinases but they may have unique substrate specificities. Therefore, different cellular targets of protein phosphorylation may account for the lack of phenotypic transformation by pp60src. It is relevant to note here that our unpublished experiments show that the level of cellular pp60src is unchanged by RSV infection, although it has been shown that the viral gene product is present in substantially greater amounts (~50-fold) in transformed cells than is the normal cell protein in uninfected cells (54, 62). Thus, cellular transformation may merely be a consequence of a quantitative difference in expression of the two genes. In that case, biochemical events in RSV-induced oncogenesis may be qualitatively identical to those in normal cells, but perhaps occur to a greater degree, as the result of pp60csrc expression, to produce the transformed phenotype. Finally, an intermediate possibility may exist such that the lower quantity of cellular pp60src is present only at a precise subcellular location restricting its activity to a limited number of substrates, whereas the greater quantity of viral pp60src with precisely the same specificity and function is distributed more widely throughout the cell, resulting in the phosphorylation of a wider variety of substrates. There is insufficient information at this time to argue strongly in support of any of the above possible explanations. Additional studies must be pursued to obtain further insights into the functional roles of pp60csrc and pp60src in neoplastic transformation and in normal cellular metabolism.
48. Radke, K., and G. S. Martin. 1979. Transformation by Rous sarcoma virus: effects of src gene expression on the synthesis and phosphorylation of cellular polypeptides. Proc. Natl. Acad. Sci. U. S. A. 76:5212-5216.

49. Erikson, E., and R. L. Erikson. 1980. Identification of a cellular protein substrate phosphorylated by the avian sarcoma virus transforming gene product. Cell 21:829-836.

50. Stehelin, D., H. E. Varmus, J. M. Bishop, and P. K. Vogt. 1976. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. Nature (Land.). 260:170-173.

51. Spector, D. H., K. Smith, T. Padgett, P. McCombe, D. Roulland-Dussoix, C. Moscovici, H. E. Varmus, and J. M. Bishop. 1978. Uninfected avian cells contain RNA related to the transforming gene of avian sarcoma virus. Cell 13:371-379.

52. Spector, D. H., B. Baker, H. E. Varmus, and J. M. Bishop. 1978. Characteristics of cellular RNA related to the transforming gene of avian sarcoma viruses. Cell. 13:381-386.

53. Wang, S. Y., W. S. Hayward, and H. Hanafusa. 1977. Genetic variation in the RNA transcripts of endogenous virus genes in uninfected chicken cells. J. Virol. 24:64-73.

54. Collett, M. S., J. S. Brugge, and R. L. Erikson. 1978. Characterization of a normal avian cell protein related to the avian sarcoma virus transforming gene product. Cell. 15:1363-1369.

55. Brugge, J. S., M. S. Collett, A. Siddiqui, B. Marczyńska, F. Deinhardt, and R. L. Erikson. 1979. Detection of the viral sarcoma protein produced in cells infected with various strains of avian sarcoma virus and of a related protein in uninfected chicken cells. J. Virol. 29:1196-1203.

56. Oppermann, H., A. D. Levintow, H. E. Varmus, L. Levintow, and J. M. Bishop. 1979. Uninfected vertebrate cells contain a protein that is closely related to the product of the avian sarcoma virus transforming gene (src). Proc. Natl. Acad. Sci. U. S. A. 76:1804-1808.

57. Kohrschneider, L. R., R. N. Eisenman, and C. R. Leitch. 1979. Identification of a Rous sarcoma virus transformation-related protein in normal avian and mammalian cells. Proc. Natl. Acad. Sci. U. S. A. 76:4479-4483.

58. Selton, B. M., T. Hunter, and K. Beemen. 1980. Relationship of polypeptide products of the transforming gene of Rous sarcoma virus and the homologous gene of vertebrates. Proc. Natl. Acad. Sci. U. S. A. 77:2059-2063.

59. Collett, M. S., E. Erikson, A. F. Purchio, J. S. Brugge, and R. L. Erikson. 1979. A normal cell protein similar in structure and function to the avian sarcoma virus transforming gene product. Proc. Natl. Acad. Sci. U. S. A. 76:3159-3163.

60. Hanafusa, H., C. C. Halpern, D. L. Buchhagen, and S. Kawai. 1977. Recovery of avian sarcoma virus from tumors induced by transformation-defective mutants. J. Exp. Med. 146:1735-1747.

61. Wang, L.-H., C. C. Halpern, M. Nadal, and H. Hanafusa. 1978. Recombination between viral and cellular sequences generates transforming sarcoma virus. Proc. Natl. Acad. Sci. U. S. A. 75:5812-5816.

62. Karase, R. E., W. S. Hayward, and H. Hanafusa. 1979. Cellular information in the genome of recovered avian sarcoma virus directs the synthesis of transforming protein. Proc. Natl. Acad. Sci. U. S. A. 76:3154-3158.