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Correlated Loss of the Transformed Phenotype and pp60^{src}-Associated Protein Kinase Activity

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Summary

The decline in pp60^{src}-associated protein kinase activity occurring after shift of Rous sarcoma virus transformation defective, mutant-infected cells was compared with changes in other parameters of transformation in an attempt to measure the relevance of the protein kinase to the overall transformed state.

Using transformation defective temperature sensitive mutants, Martin (1970) presented the first evidence that a Rous sarcoma viral gene product was continuously needed for maintainance of transformation. More recently, Ash et al. (1976) showed that a reversible return to a normal phenotype could be induced in Rous sarcoma virus transformed cells by treatment with inhibitors of protein synthesis. This was interpreted to mean that a viral gene product directly or indirectly induces the transformed state and that transformed cells, freed of this effector because of its presumably modest half-life in the cell, take on spontaneously a normal phenotype without requirement for new protein synthesis. This communication wil document a similar reversion in the transformed phenotype, induced in transformation-defective, temperature-sensitive, mutant-infected cells after a shift from the permissive to the nonpermissive temperature.

The *src* gene of Rous sarcoma virus apparently is entirely and uniquely responsible for inducing transformation (Wang et al. 1975). Brugge and Erikson (1977) recently demonstrated that the gene product of the *src* gene, called pp60^{src}, was a phosphoprotein of 60,000 daltons which could be immunoprocipitated using tumor-bearing rabbit serum. Furthermore, a protein kinase activity capable of phosphorylating the immune precipitating IgG was detected (Collett and Erikson 1978) and is apparently associated with the pp60^{src}. It seems also highly probable that the pp60^{src} also acts autocatalytically to phosphorylate one site on its own molecule.

Figure 1 illustrates an experiment in which mutant (A) and wild type (B) infected cells which had been grown at 35°C (permissive) were shifted and assayed at various times after shift to 42°C (nonpermissive). A kinase reaction is shown in the first half of each polyacrylamide gel and the intensity of the IgG heavy chain serves as a measure of kinase activity. The second half of the gel presents an immunoprecipitation of in vivo orthophosphate (^{32}P)-labeled cell lysates with the pp60^{src} band indicated. In looking at kinase activity it is clear in comparing A and B that the mutant kinase is sharply thermolabile; a loss of 5-fold in activity is measured within 30 min. This is not seen with the Schmidt-Ruppin strain wild type parent virus-infected cells which retain approximately constant kinase activity after temperature shift. As with kinase activity, ^{32}P-orthophosphate incorporated into pp60^{src} in a pulse label at 35°C is rapidly lost in a chase at 42°C for the mutant (A), but is lost somewhat more slowly with the wild type virus-infected cells (B). Hence, the rapid loss of kinase activity seen with the mutant-infected cells upon temperature shift to 42°C correlates with a dephosphorylation occurring more rapidly than the half life of the incorporated phosphate in the cells infected by the wild type virus could account for.

Parallel studies examined the change in the rate of hexose transport shown by mutant-infected cells upon shift to the nonpermissive temperature. Within 4 h after the shift to 42°C,

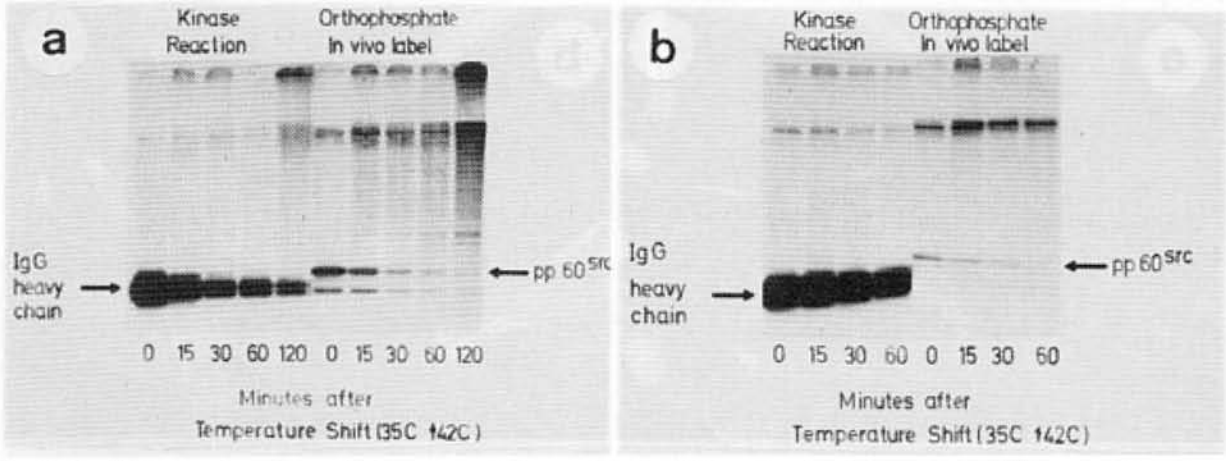

Fig. 1. Pp60^{src}-associated protein kinase activity and the half life of in vivo incorporated ^{32}P-orthophosphate are shown. The kinase reaction was performed as already described (Rübsamen et al. 1980). For the half life study, cultures were labeled for 20 min at 35°C with 1 mCI/ml of ^{32}P-orthophosphate, followed by a chase according to the times indicated at 42°C. **a** shows results obtained with NY68 (Kawai and Hanafusa 1971) infected cells, while **b** was transformed with the Schmidt Ruppin strain of Rous sarcoma virus wild type parent. *Arrows* indicate the heavy chain of IgG and pp60^{src}

cells showed a 50% reduced rate of uptake of ^{3}H-2-deoxyglucose in a 10 min incubation when compared to sister cultures maintained at 35°C.

Figure 2 illustrates the changes occurring in the organization of mutant-infected cell microfilaments concomitant with a temperature shift from 35°C to 42°C. The method used takes advantage of the high affinity binding of the fluorescent-labeled toxin, phalloidin (the generous gift of Prof. Th. Wieland, Heidelberg), to filamentous actin (Wieland and Faulstich 1978). The initial conditions (35°C) is shown in the first picture (a); all cells show few stress fibers, and several (the central cell and several to the right side) show virtually no stress fibers, with diffuse and punctate fluorescence. In pucture (b), taken just 30 min after the shift to 42°C, most cells show better outlined stress fibers and some degree of linear array in all cells. Figure 2 (c) shows notably a cell with an apparently regular punctate fluorescence and developing stress fibers array at 60 min after temperature shift. Finally, 4 h after shift to 42°C, all cells (d) display well developed stress fiber arrays.

The data presented above indicate how rapidly the change in phenotype from transformed to normal can occur. Such kinetics have been previously examined, but heretofore always using cells in the normal phenotype at 42°C shifted to 35°C in order to observe the onset of transformation (Ziemiecki and Friis, to be published). In the present study, as in the work of Ash et al. (1976), the interesting finding is that upon loss of the *src* gene product's activity, here monitored as the associated protein kinase, the cell undergoes a rapid change to normal. Ash et al. (1976) established that this change took place without the need of new protein synthesis; their result was obtained only 12–16 h after treatment, owing to the rather long functional life of the *src* gene product under these conditions. Using mutants, in which case the loss of *src* gene function is very rapid, and without using inhibitors which might have morphological effects of their own, the same result emerged from our investigation.

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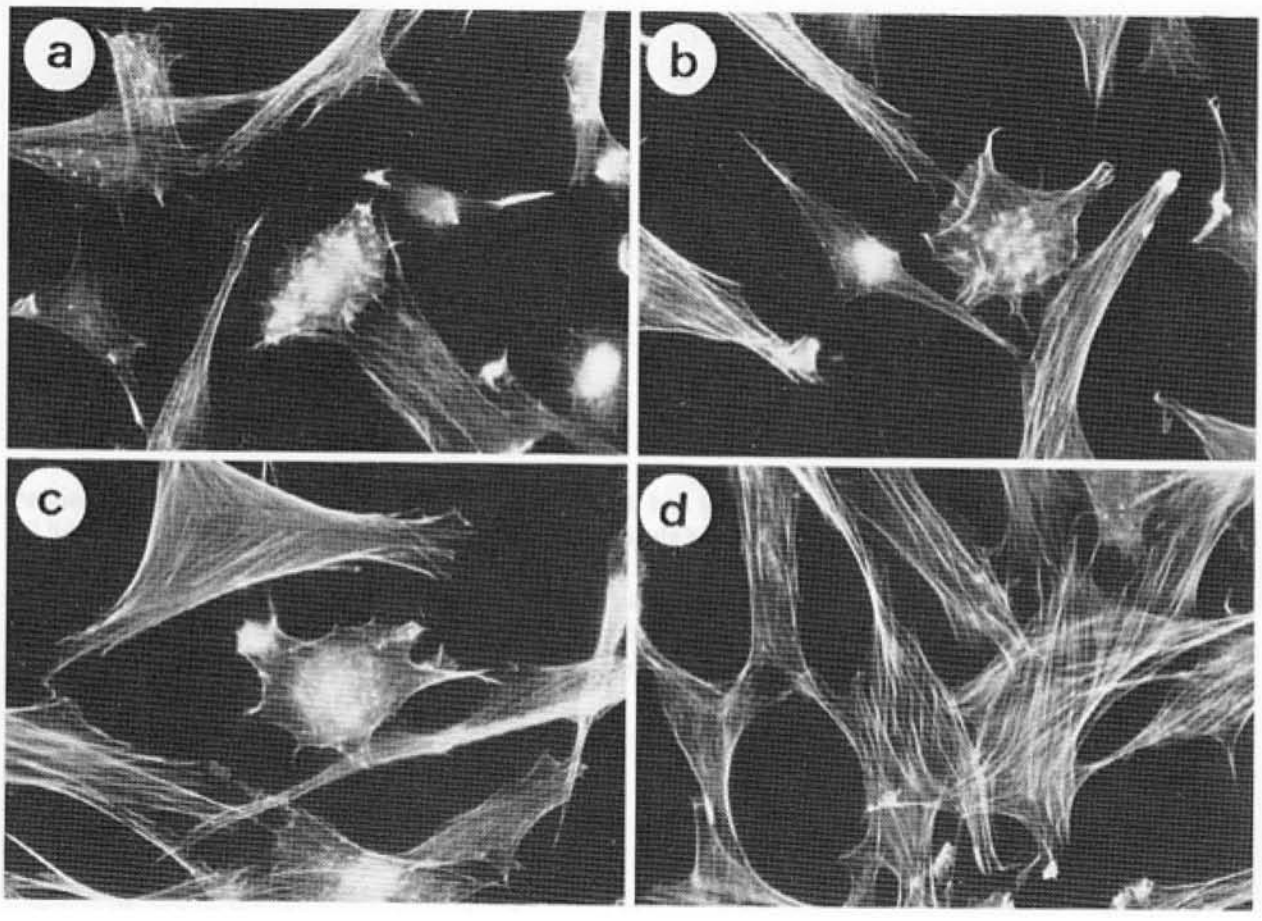

Fig. 2. Photomicrographs of NY68 infected cells fixed with 2.5% formaldehyde containing 0.2% Triton X-100 and stained with fluorescein isothiacyanate labeled phalloidin for 30 min at 25°C. Cells were prepared rapidly at the following conditions: **a** 35°C; **b** shifted to 42°C for 30 min, **c** shifted to 42°C for 60 min, and **d** shifted to 42°C for 4 h. Final magnification 592×

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