MOLECULAR AND CELLULAR BIOLOGY, Apr. 1982, p. 355–360
0270-7306/82/040355-06$02.00/0

Vol. 2, No. 4

Half-Life of the Rous Sarcoma Virus Transforming Protein pp60^{src} and Its Associated Kinase Activity

ANDREW ZIEMIECKI,* ROBERT R. FRIIS, AND HEINZ BAUER

Institut für Virologie, Fachbereich Humanmedizin der Justus-Liebig-Universität, 6300 Giessen, West Germany

Received 29 September 1981/Accepted 24 November 1981

The half-life of metabolically labeled pp60^{src} of the Prague A strain of Rous sarcoma virus and of several transformation-defective, temperature-sensitive mutants was investigated by pulse-labeling infected cells with [^{35}S]methionine, chasing for different times, and immunoprecipitating pp60^{src} with tumor-bearing rabbit serum. These experiments showed that pp60^{src} has a short half-life of approximately 60 min under normal physiological conditions and that the mutant pp60^{src} proteins have similar half-lives to the wild type, irrespective of whether the cells are kept at the nonpermissive (42°C) or permissive (35°C) temperature. The half-life of the pp60^{src}-associated kinase activity was determined by monitoring its decay by the immunoglobulin G heavy chain assay after the cells had been treated with several inhibitors of protein synthesis. In these experiments the kinase half-life was much longer than expected from the half-life of pp60^{src}. The apparent contradiction between the half-lives of the kinase activity and the [^{35}S]methionine-labeled pp60^{src} protein could be resolved by the observation that treatment of cells with inhibitors of protein synthesis stabilized pp60^{src}, resulting in a greatly extended half-life. Inhibitors of protein synthesis also extended the half-life of the *gag* precursor polypeptide, Pr76, suggesting that a host factor(s) may be required for the efficient intracellular processing of this polypeptide to the *gag* proteins.

The continual expression of the *src* gene of Rous sarcoma virus (RSV) is required for maintenance of the transformed phenotype in infected cells (19). A 60,000-dalton phosphoprotein, pp60^{src}, has been shown to be a product of the *src* gene (4, 5, 18), and a protein kinase activity capable of phosphorylating in vitro several substrates at tyrosine has been demonstrated to be intimately associated with pp60^{src} (6, 7, 10, 13). Despite extensive biochemical investigation of pp60^{src}, little is known about the in vivo half-life of the molecule itself and its associated kinase activity (25). In this paper we describe experiments aimed at determining the half-lives of metabolically labeled pp60^{src} and the associated protein kinase activity. We show that pp60^{src} metabolically labeled with [^{35}S]methionine has a short half-life of approximately 60 min. In experiments in which the decay of the pp60^{src}-associated kinase activity was followed in cells treated with cycloheximide and other inhibitors of protein synthesis, a long half-life of many hours was observed. These results could be reconciled by our observation that the protein synthesis inhibitors used to demonstrate the decay of pp60^{src}-associated kinase activity greatly prolonged the half-life of metabolically labeled pp60^{src}.

MATERIALS AND METHODS

Cells and viruses. Chicken embryo cell cultures were prepared with 10-day-incubated specific-pathogen-free White Leghorn eggs, the generous gift of E. Vielitz (Lohmann-Tierzucht, Cuxhaven, West Germany). Cultures were maintained in Dulbecco modified Eagle minimal essential medium supplemented with 5% newborn calf serum and 10% tryptose phosphate broth. For radioactive labeling, [^{35}S]methionine (670 Ci/mmol; Amersham Buchler, Braunschweig, West Germany) was used at 100 μCi/ml in medium completely lacking methionine. The following inhibitors of protein synthesis were used: cycloheximide at 10 μg/ml, emetine at 25 μg/ml, puromycin at 20 μg/ml, and cordycepin at 25 μg/ml. Tunicamycin, an inhibitor of glycosylation, was used at 1 μg/ml. Excepting tunicamycin, which was the generous gift of Ralph Schwarz, Giessen, all drugs were obtained from Sigma Chemical Co. (Taufkirchen, West Germany).

The Prague strain of RSV, subgroup A, was the parent of the transformation-defective, temperature-sensitive mutants GI203, GI251, and GI253 (2). The Schmidt-Ruppin strain of RSV, subgroup A, was the parent of the transformation-defective, temperature-sensitive mutant NY68 (14). Rous-associated virus type 1, an avian leukosis virus, was used as a control in these experiments for a virus lacking the *src* gene.

Cell lysis, immunoprecipitation, kinase assay, and electrophoresis. [^{35}S]methionine-labeled cells used for the pulse-chase experiments were lysed in RIPA buffer

(10 mM PO_4 [pH 7.2], 10 mM EDTA, 40 mM NaF, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and 5% Trasylol) and clarified by centrifugation in an Eppendorf centrifuge. The supernatant was incubated for 60 min on ice with rabbit antiserum raised against the viral structural proteins which had been adsorbed onto protein A-bearing *Staphylococcus aureus* bacteria (Cowan I strain). (This preincubation step was used only in the experiments shown in Fig. 1 and 2 and served to reduce the background in the immunoprecipitation patterns.) After preincubation, the bacteria were pelleted, and the supernatant was incubated for 60 min on ice with an excess of tumor-bearing rabbit (TBR) serum. Details of TBR serum preparation have been published elsewhere (30). Immune complexes were isolated by adsorption onto protein A-bearing *S. aureus* bacteria in RIPA buffer (16). Bacteria-bound immune complexes were washed three times with wash buffer (10 mM PO_4 [pH 7.2], 10 mM EDTA, 40 mM NaF, 1 M NaCl, 1% Triton X-100, and 5% Trasylol) and finally once with water. Radiolabeled proteins were eluted from the bacteria by suspending the bacterial pellets in electrophoresis sample buffer (80 mM Tris [pH 6.8], 10% glycerol, 2% sodium dodecyl sulfate, and 2% mercaptoethanol) and boiling for 2 min. Polypeptides were resolved on 6 to 18% acrylamide gradient gels (17).

The $pp60^{src}$-associated kinase activity was assayed essentially as previously reported (21). Cells were lysed in Triton buffer (10 mM PO_4 [pH 7.2], 10 mM EDTA, 40 mM NaF, 1% Triton X-100, and 5% Trasylol) and clarified, and the supernatant was incubated on ice with TBR serum for 60 min. Immune complexes were isolated by adsorption onto protein A-bearing *S. aureus* bacteria in Triton buffer and washed as described above. Bacteria loaded with antigen-antibody complexes were resuspended in a small volume of kinase buffer (20 mM bis-Tris-propane [pH 6.0] and 50 mM $MgCl_2$), thoroughly mixed with [γ-^{32}P]ATP (0.5 to 1 μM, 3,000 Ci/mmol; Amersham Buchler), and incubated for 5 min on ice. The reaction was terminated by the addition of quench buffer (100 mM PO_4 [pH 7.0], 10 mM EDTA, 40 mM NaF, and 1 mM ATP). Bacterium-antigen-antibody complexes were pelleted and resuspended in electrophoresis sample buffer. The phosphorylated heavy chain of immunoglobulin G was resolved on 11% acrylamide gels (17).

Radioactively labeled bands were detected by fluorography (3). [^{35}S]methionine-labeled bands were additionally quantitated by cutting the desired bands out, reswelling in Lumasolve (J. T. Baker Chemical Co., Gross Gerau, West Germany), and after the addition of scintillation fluid, counting in a scintillation counter. ^{32}P-labeled heavy chains were cut out after Coomassie brilliant blue staining, and the Cerenkov radiation was determined.

RESULTS

Half-life of metabolically labeled $pp60^{src}$. Infected, transformed sister cultures of chicken fibroblasts were pulse-labeled for 15 min with [^{35}S]methionine, chased for different times, and lysed with RIPA buffer. Clarified lysates were preabsorbed with rabbit antiserum raised against viral structural proteins, and then $pp60^{src}$ was immunoprecipitated with TBR serum. The precipitated [^{35}S]methionine-labeled proteins were resolved on polyacrylamide gels, and the $pp60^{src}$ band was located, cut out, and quantitated by scintillation counting. Results of such an experiment are shown in Fig. 1 and 2. The Prague A strain $pp60^{src}$ has a relatively short half-life of approximately 60 min irrespective of the temperature at which the cells were grown. Essentially the same results were obtained if the cells were labeled overnight (20 h) and then chased.

We also investigated the half-lives of the $pp60^{src}$ proteins of several transformation-defective, temperature-sensitive mutants grown at either the permissive (35°C) or the nonpermissive (42°C) temperature. Figure 3 shows a composite of the relevant sections of two gels containing the mutant $pp60^{src}$ bands. Wild-type Prague A- and Rous-associated virus type 1-infected cells acted as controls. From the figure it can be concluded that the half-life of the mutant $pp60^{src}$ proteins is similar to that of the wild type, and there is little difference in the mutant $pp60^{src}$ half-lives at 42 and 35°C. These conclusions are supported by the quantitative data shown in Fig. 2 for one of the mutants, GI251.

Half-life of the $pp60^{src}$-associated kinase activity. A convenient way to determine the half-life of the $pp60^{src}$-associated kinase activity is to monitor its decay in cells treated with inhibitors of protein synthesis. Accordingly, infected, transformed sister cultures were treated with cycloheximide, emetine, cordycepin, or puromycin, and the decay with time of the $pp60^{src}$-

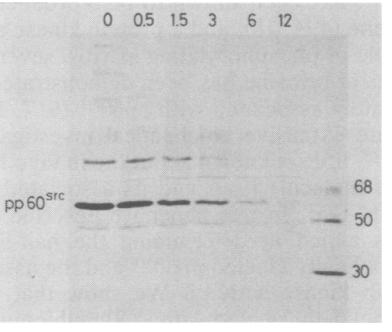

FIG. 1. The half-life of [^{35}S]methionine-labeled $pp60^{src}$ of the Prague A strain of RSV. Sister cultures were pulse-labeled for 15 min at 42°C and chased for the times indicated (in hours) at the top of the figure. Cells were lysed in RIPA buffer, clarified, and preabsorbed with antiserum against viral structural proteins, and $pp60^{src}$ was precipitated with TBR serum. The labeled proteins were resolved on 6 to 18% acrylamide gradient gels and detected by fluorography. Semliki Forest virus structural proteins pE2 (68,000), E1 (50,000), and C (30,000) acted as molecular weight standards.

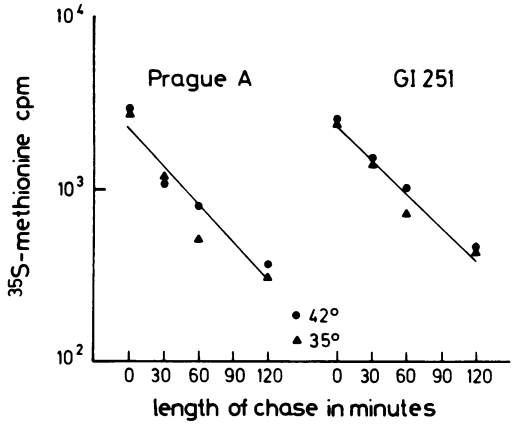

FIG. 2. The half-life of [^{35}S]methionine-labeled pp60^{src} of the Prague A strain of RSV and of the transformation-defective, temperature-sensitive mutant GI251 at the nonpermissive temperature (42°C) and the permissive temperature (35°C). Infected sister cultures kept at either 42 or 35°C were pulse-labeled for 15 min, chased for the times indicated, and processed as in Fig. 1. The pp60^{src} was quantitated by scintillation counting of excised bands.

associated kinase activity was measured by the immunoglobulin G heavy chain kinase assay (6). The concentrations of the inhibitors used gave better than 98% inhibition of host cell protein synthesis as judged by incorporation of [^{35}S]methionine into polypeptides (data not shown). Cell lysates were assayed either undiluted (as in Fig. 4) or diluted 1:10 with Triton buffer to ensure antibody excess. As can be seen in Fig. 4, under a regime of protein synthesis inhibition, the half-life of the pp60^{src}-associated protein kinase activity was very much longer than we had expected from the half-life of metabolically labeled pp60^{src} (cf. Fig. 4 with Fig. 1 and 2). Similar results were obtained with the diluted extracts. The decay in kinase activity in cells treated with puromycin was consistently more extensive than that seen with other inhibitors.

Inhibitors of protein synthesis prolong the half-life of metabolically labeled pp60^{src}. The results documented above presented us with a dilemma. On the one hand, the metabolically labeled pp60^{src} clearly had a short half-life, and on the other hand, the associated kinase activity had a much longer half-life. We suspected that our experimental procedure for determining the kinase half-life was giving artifactual results. We therefore decided to see if treatment of cells with inhibitors of protein synthesis had any effect on the half-life of metabolically labeled pp60^{src}. Infected, transformed sister cultures were pulse-labeled and chased for different times either in the absence of (control) or in the presence of cycloheximide, and the half-life of metabolically labeled pp60^{src} was determined. The results of such an experiment are shown in Fig. 5. Drug treatment greatly prolonged the half-life of pp60^{src}. Other inhibitors of protein synthesis (emetine, cordycepin, puromycin) had a very similar effect on the pp60^{src} half-life. That the band in question is pp60^{src} was confirmed in a parallel experiment by either preabsorbing the cell extracts with antiserum raised against the viral structural proteins or preabsorbing the TBR serum with solubilized purified virus. Interestingly, the half-life of the viral *gag* gene product Pr76 was also prolonged in a parallel manner (Fig. 5). In untreated cells this precursor molecule is rapidly processed to the *gag* proteins. Another uncharacterized protein of high molecular weight which coprecipitated with pp60^{src} and Pr76 (Pr180?) also showed a similar prolongation of its half-life (asterisk on Fig. 5). If after a 3-h chase in the presence of cycloheximide the drug was removed and the chase was continued for 2 h (Fig. 5, lane 3–5) or 6 h (Fig. 5, lane 3–9) in the absence of the drug, processing

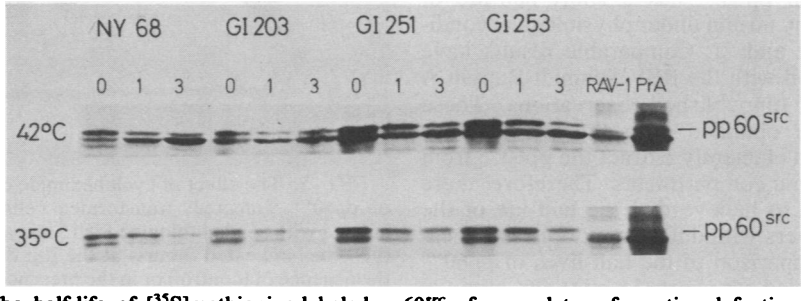

FIG. 3. The half-life of [^{35}S]methionine-labeled pp60^{src} of several transformation-defective, temperature-sensitive mutants of RSV at the nonpermissive temperature (42°C) and the permissive temperature (35°C). Sister cultures infected with the various mutants and kept at either 42 or 35°C were pulse-labeled for 15 min, chased for the times indicated (hours), and processed as in Fig. 1 except that the preabsorption with antiserum against virus structural proteins was omitted. The pp60^{src} of the Schmidt-Ruppin mutant strain NY68 is slightly smaller than that of the Prague A strain (PrA) mutants GI203, GI251, and GI253. RAV-1, Rous-associated virus type 1.

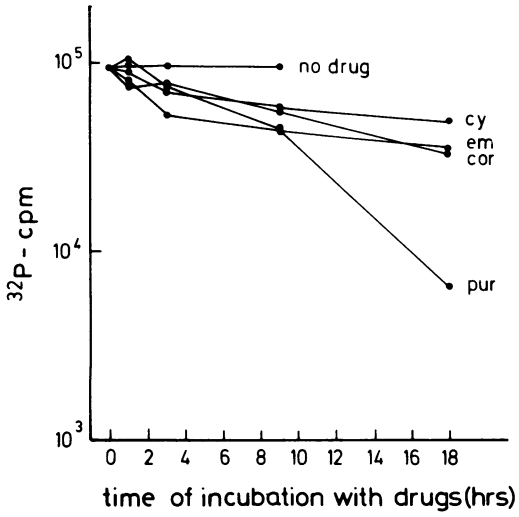

FIG. 4. The half-life of the pp60^{src}-associated kinase activity. Sister cultures of cells infected and transformed with the Schmidt-Ruppin A strain of RSV were treated with various inhibitors of protein synthesis (cy, cycloheximide; em, emetine; cor, cordycepin; pur, puromycin), and the decay in kinase activity was monitored by the immunoglobulin G heavy chain assay (6). After electrophoresis, the heavy chains were visualized by Coomassie brilliant blue staining, excised, and counted in a scintillation counter.

of pp60^{src}, Pr76, and the uncharacterized high-molecular-weight protein was restored.

DISCUSSION

The RSV transforming protein, the pp60^{src}, has been widely supposed to be relatively stable, with a half-life of many hours in the cell. This conclusion is implicit in the work of Ash et al. (1) where the gradual loss of the transformed phenotype from RSV-infected cells over a period of 12 to 16 h under the influence of cycloheximide was reported. Our results indicate that the Prague strain pp60^{src} has a short half-life of approximately 60 min under physiological conditions (Fig. 1 and 2). Comparable results have been obtained with the RSV Schmidt-Ruppin A strain pp60^{src} (unpublished observations). These results were obtained with the RIPA buffer which should efficiently extract the pp60^{src} from all cytoplasmic compartments. Therefore, there is no reason to believe that the half-life of the molecule differs with different cytoplasmic locations. A comparison of the half-lives of pp60^{src} proteins labeled for short (15 min) and long (20 h) times with [^{35}S]methionine has also been performed, and no differences were observed. Hence, it seems unlikely that pp60^{src} exists as two different populations, one long-lived. Finally, several well-characterized, temperature-sensitive mutants of RSV were examined for the half-life of pp60^{src} at permissive (35°C) and nonpermissive (42°C) temperatures (Fig. 3). The half-lives of the mutant proteins differed little from the wild type and differed little at 35 and 42°C. These results are consistent with our previous observations on the amounts of pp60^{src} present at 35 and 42°C in cells infected with these temperature-sensitive mutants (22). Sefton et al. (25), using metabolic labeling methods, have documented that the pp60^{src} of the Schmidt-Ruppin D strain is metabolically stable, with a half-life in excess of 2 h. The discrepancy between our results, primarily with the Prague A strain, and those of Sefton et al. (25) may reflect either differences in the stability of the pp60^{src} of different strains or the state of health of the cell or a combination of both.

Measurement of the half-life of the pp60^{src}-associated protein kinase activity can be performed conveniently only by following its decay in cells treated with inhibitors of protein synthesis. With several inhibitors of protein synthesis, the half-life of the pp60^{src}-associated kinase activity was found to be much longer than the half-life of metabolically labeled pp60^{src} (cf. Fig. 2 and 4). A likely resolution to this contradiction is offered by the observation that metabolically labeled pp60^{src} is stabilized in cells which have been treated with cycloheximide, this stabilization resulting in a greatly extended half-life (Fig. 5). That the inhibitor has this effect is substanti-

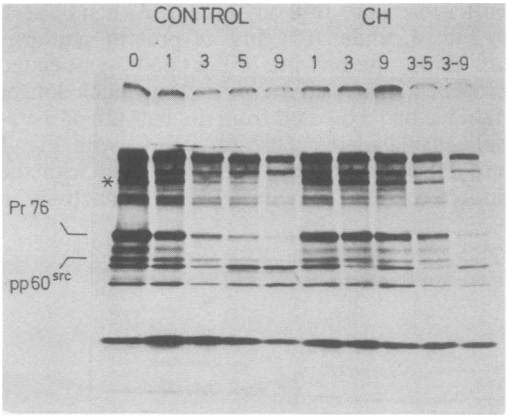

FIG. 5. The effect of cycloheximide on the half-life of pp60^{src}. Infected, transformed cells were pulse-labeled with [^{35}S]methionine for 15 min and chased for the times indicated (hours) at the top of the figure in the absence of (control) or in the presence of cycloheximide (CH). The cells were processed as for Fig. 3. The last two lanes represent cells which were pulse-labeled and chased for 3 h in the presence of cycloheximide and then for a further 2 h (3–5) or 6 h (3–9) in the absence of cycloheximide. *, An uncharacterized high-molecular-weight protein, possibly Pr180.

ated by the observation that when cycloheximide is removed 3 h after beginning the chase and the cultures are chased further in medium lacking cycloheximide, the rapid turnover of pp60^{src} can be reestablished (Fig. 5, lanes 3–5 and 3–9). Similar results were obtained with emetine, puromycin, and cordycepin. Tunicamycin, an inhibitor of glycosylation, had no effect on the pp60^{src} half-life (unpublished observations).

Thus, it seems that pp60^{src} is in all probability a protein with a short half-life in the chicken cell, a conclusion consistent with a regulatory function. For comparison, the total cellular protein in chicken embryo fibroblasts is reported to have a half-life of 40 to 50 h (12, 29). The rather slow reestablishment of normal cell phenotype observed by Ash et al. (1) after cycloheximide treatment was a consequence of the extended half-life conferred upon pp60^{src} by the cycloheximide. Also, our observation that mutant-infected cells underwent morphological transformation over a period of up to 6 h after a shift to the permissive temperature in the presence of cycloheximide (11) must be attributed to the stabilizing influence of the drug.

The observation that the half-life of Pr76 is also prolonged after cycloheximide treatment (Fig. 5) raises the possibility that a host factor(s) may be involved in its processing to the viral *gag* proteins. In vitro experiments with purified Pr76 and p15 have shown that p15 alone is capable of proteolytically processing Pr76 (8, 28); however, the in vivo processing, taking place at much lower p15 concentrations, may involve a cooperation between p15 and an as yet uncharacterized host factor(s).

In comparison with the information available on the synthesis of proteins, very little is known about how proteins are degraded in the cell (24). The observed stabilization of pp60^{src} and Pr76 by cycloheximide may reflect a part of the cell's protein-degrading system, conceivably, continually synthesized short-lived proteases. Several examples of the stabilizing effect of inhibitors of protein synthesis on enzyme activities have been reported (9, 12, 15, 26, 27). However, such postulated short-lived proteases are only a part of the cell's degrading system since there are other examples where inhibitors of protein synthesis have no stabilizing effect on enzyme activities (20, 23).

ACKNOWLEDGMENTS

We thank E. Vielitz, Lohmann-Tierzucht, Cuxhaven, West Germany, for the regular supply of specific-pathogen-free chicken eggs and Siggi Bingel for excellent technical assistance.

This work was supported by the Sonderforschungsbereich 47 (Virologie) of the Deutsche Forschungsgemeinschaft.

LITERATURE CITED

1. **Ash, J. F., P. K. Vogt, and S. J. Singer.** 1976. Reversion from transformed to normal phenotype by inhibition of protein synthesis in rat kidney cells infected with a temperature-sensitive mutant of Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **73**:3603–3607.
2. **Becker, D., R. Kurth, D. Critchley, R. R. Friis, and H. Bauer.** 1977. Distinguishable transformation-defective phenotypes among temperature-sensitive mutants. J. Virol. **21**:1042–1055.
3. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
4. **Brugge, J. S., E. Erikson, M. S. Collett, and R. L. Erikson.** 1978. Peptide analysis of the transformation-specific antigen from avian sarcoma virus-transformed cells. J. Virol. **26**:773–782.
5. **Brugge, J. S., and R. L. Erikson.** 1977. Identification of a transformation-specific antigen induced by an avian sarcoma virus. Nature (London) **269**:346–348.
6. **Collett, M. S., and R. L. Erikson.** 1978. Protein kinase associated with the avian sarcoma virus src gene product. Proc. Natl. Acad. Sci. U.S.A. **75**:2021–2024.
7. **Collett, M. S., A. F. Purchio, and R. L. Erikson.** 1980. Avian sarcoma virus-transforming protein, pp60^{src}, shows protein kinase activity specific for tyrosine. Nature (London) **285**:167–169.
8. **Dittmar, K. J., and K. Moelling.** 1978. Biochemical properties of p15-associated protease in an avian RNA tumor virus. J. Virol. **28**:106–118.
9. **Epstein, D., S. Elias-Bishko, and A. Hershko.** 1975. Requirement for protein synthesis in the regulation of protein breakdown in cultured hepatoma cells. Biochemistry **14**:5199–5204.
10. **Erikson, R. L., M. S. Collett, E. Erikson, A. F. Purchio, and J. S. Brugge.** 1979. Protein phosphorylation mediated by partially purified avian sarcoma virus transforming-gene product. Cold Spring Harbor Symp. Quant. Biol. **44**:907–917.
11. **Friis, R. R., B. M. Jockusch, C. B. Boschek, A. Ziemiecki, H. Rübsamen, and H. Bauer.** 1979. Transformation-defective, temperature-sensitive mutants of Rous sarcoma virus have a reversibly defective *src*-gene product. Cold Spring Harbor Symp. Quant. Biol. **44**:1007–1012.
12. **Hendil, K. B.** 1977. Intracellular protein degradation in growing, in density-inhibited, and in serum-restricted fibroblast cultures. J. Cell. Physiol. **92**:353–364.
13. **Hunter, T., and B. M. Sefton.** 1980. Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. Proc. Natl. Acad. Sci. U.S.A. **77**:1311–1315.
14. **Kawai, S., and H. Hanafusa.** 1971. Effects of reciprocal changes in temperature on the transformed state of cells infected with a Rous sarcoma virus mutant. Virology **46**:470–479.
15. **Kenney, F. T.** 1967. Turnover of rat liver tyrosine transaminase: stabilization after inhibition of protein synthesis. Science **156**:525–528.
16. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with a Staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115**:1617–1624.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
18. **Levinson, A. D., H. Oppermann, L. Levintow, H. E. Varmus, and J. M. Bishop.** 1978. Evidence that the transforming gene of avian sarcoma virus encodes a protein kinase associated with a phosphoprotein. Cell **15**:561–572.
19. **Martin, G. S.** 1970. Rous sarcoma virus: a function required for the maintenance of the transformed state. Nature (London) **227**:2021–2023.
20. **Marver, H. S., D. P. Tschudy, M. G. Perlroth, and A. Collins.** 1966. δ-Aminoevulinic acid synthetase. J. Biol.

Chem. 241:2803–2809.
21. **Rübsamen, H., R. R. Friis, and H. Bauer.** 1979. *Src* gene product from different strains of Rous sarcoma virus: kinetics and possible mechanism of heat inactivation of protein kinase activity from cells infected by transformation-defective, temperature-sensitive mutant and wild-type virus. Proc. Natl. Acad. Sci. U.S.A. 76:967–971.
22. **Rübsamen, H., A. Ziemiecki, R. R. Friis, and H. Bauer.** 1980. The expression of $pp60^{src}$ and its associated protein kinase activity in cells infected with different transformation-defective temperature-sensitive mutants of Rous sarcoma virus. Virology 102:453–457.
23. **Schimke, R. T.** 1967. Enzymes of arginine metabolism in cell cultures: studies on enzyme induction and repression. Natl. Cancer Inst. Monogr. 27:301–309.
24. **Schimke, R. T., and D. Doyle.** 1970. Control of enzyme levels in animal tissues. Annu. Rev. Biochem. 39:929–976.
25. **Sefton, B. M., K. Beemon, and T. Hunter.** 1978. Comparison of the expression of the *src* gene of Rous sarcoma virus in vitro and in vivo. J. Virol. 28:957–971.
26. **Sellinger, P. Z., K. L. Lee, and K. M. Fesler.** 1966. The induction of mitochondrial α-gylycerophosphate dehydrogenase by thyroid hormone: effects of adrenalectomy, thyroidectomy, and cortisone administration. Biochim. Biophys. Acta 124:289–294.
27. **Shambaugh, G. E., J. B. Balovsky, and P. O. Cohen.** 1969. Synthesis of carbonyl phosphate synthetase in amphibian liver *in vitro*: the effect of thyroxine. J. Biol. Chem. 244:5295–5308.
28. **Von der Helm, K.** 1977. Cleavage of Rous sarcoma viral polypeptide precursor into internal structural proteins *in vitro* involves viral protein p15. Proc. Natl. Acad. Sci. U.S.A. 74:911–915.
29. **Weber, M. J.** 1972. Ribosomal RNA turnover in contact inhibited cells. Nature (London) New Biol. 235:58–61.
30. **Ziemiecki, A., and R. R. Friis.** 1980. Simultaneous injection of newborn rabbits with the Schmidt-Ruppin and Prague strains of Rous sarcoma virus induces antibodies which recognize the $pp60^{src}$ of both strains. J. Gen. Virol. 50:211–216.