Video Article

Analysis of Protein Folding, Transport, and Degradation in Living Cells by Radioactive Pulse Chase

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Abstract

Radioactive pulse-chase labeling is a powerful tool for studying the conformational maturation, the transport to their functional cellular location, and the degradation of target proteins in live cells. By using short (pulse) radiolabeling times (<30 min) and tightly controlled chase times, it is possible to label only a small fraction of the total protein pool and follow its folding. When combined with nonreducing/reducing SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoprecipitation with (conformation-specific) antibodies, folding processes can be examined in great detail. This system has been used to analyze the folding of proteins with a huge variation in properties such as soluble proteins, single and multi-pass transmembrane proteins, heavily N- and O-glycosylated proteins, and proteins with and without extensive disulfide bonding. Pulse-chase methods are the basis of kinetic studies into a range of additional features, including co- and posttranslational modifications, oligomerization, and polymerization, essentially allowing the analysis of a protein from birth to death. Pulse-chase studies on protein folding are complementary with other biochemical and biophysical methods for studying proteins *in vitro* by providing increased temporal resolution and physiological information. The methods as described within this paper are adapted easily to study the folding of almost any protein that can be expressed in mammalian or insect-cell systems.

Video Link

The video component of this article can be found at https://www.jove.com/video/58952/

Introduction

The folding of even relatively simple proteins involves many different folding enzymes, molecular chaperones, and covalent modifications[1]. A complete reconstitution of these processes *in vitro* is practically impossible, given the vast number of different components involved. It is highly desirable, therefore, to study protein folding *in vivo*, in live cells. Radioactive pulse-chase techniques prove a powerful tool for studying the synthesis, folding, transport, and degradation of proteins in their natural environment.

The metabolic labeling of proteins during a short pulse with ^{35}S-labeled methionine/cysteine, followed by a chase in the absence of a radioactive label, allows specific tracking of a population of newly synthesized proteins in the wider cellular milieu. Then, target proteins can be isolated *via* immunoprecipitation and analyzed *via* SDS-PAGE or other techniques. For many proteins, their journey through the cell is marked by modifications that are visible on SDS-PAGE gel. For example, the transport of glycosylated proteins from the endoplasmic reticulum (ER) to the Golgi complex is often accompanied by modifications of N-linked glycans or the addition of O-linked glycans[2,3]. These modifications cause large increases in the apparent molecular mass, which can be seen by mobility changes in SDS-PAGE. Maturation can also be marked by proteolytic cleavages, such as signal-peptide cleavage or the removal of pro-peptides, resulting in changes in the apparent molecular mass that can be followed easily on SDS-PAGE gel[4]. Radioactivity has considerable advantages over comparable techniques such as cycloheximide chases, where novel protein synthesis is prevented, as longer treatments are toxic to cells and do not exclude the majority of older, steady-state proteins from the analysis, as some proteins have half-lives of days. The comparison of proteins under both nonreducing and reducing conditions allows the analysis of disulfide bond formation, an important step in the folding of many secretory proteins[4,5,6,7].

Here we describe a general method for the analysis of protein folding and transport in intact cells, using a radioactive pulse-chase approach. While we have aimed to provide the method as detailed as possible, the protocol has an almost limitless potential for adaptability and will allow optimization to study each reader's specific proteins.

Two alternative pulse-chase protocols, one for adherent cells (step 1.1 of the protocol presented here) and one for suspension cells (step 1.2 of the protocol presented here) are provided. The conditions provided here are sufficient to visualize a protein expressed with medium- to high-expression levels. If the reader is working with poorly expressed proteins or various posttreatment conditions, such as multiple immunoprecipitations, it is necessary to increase the dish size or cell number appropriately.

For suspension pulse chase, the chase samples taken at each time point are all taken from a single tube of cells. The wash steps after the pulse are omitted; instead, further incorporation of ^{35}S is prevented by dilution with a high excess of unlabeled methionine and cysteine.

The presented protocols use radioactive ^{35}S-labeled cysteine and methionine to follow cellular protein-folding processes. All operations with radioactive reagents should be performed using appropriate protective measures to minimize any exposure of the operator and the environment to radioactive radiation and be performed in a designated laboratory. As the pulse-chase labeling technique is relatively inefficient at short pulse times (<15 min), less than 1% of the starting amount of radioactivity is incorporated in the newly synthesized proteins. After the enrichment of the target protein *via* immunoprecipitation, the sample for SDS-PAGE contains less than 0.05% of the starting amount of radioactivity.

Although the ^{35}S methionine and cysteine labeling mix is stabilized, some decomposition, yielding volatile radioactive compounds, will occur. To protect the researcher and the apparatus, some precautions should be taken. The researcher should always obey the local radiation safety rules and may wear a charcoal nursing mask, besides a lab coat and (double) gloves. Stock vials with ^{35}S methionine and cysteine should always be opened in a fume hood, or under a local aspiration point. Known laboratory contamination spots are centrifuges, pipettes, water baths, incubators, and shakers. The contamination of these areas is reduced by using pipette tips with a charcoal filter, positive-seal microcentrifuge tubes (see **Table of Materials**), aquarium charcoal sponges in water baths, charcoal filter papers glued in the pulse-chase dishes, charcoal guard in the aspiration system, and the placement of dishes containing charcoal grains in incubators and storage containers.

Protocol

All radioactive reagents and procedures were handled in accordance with local Utrecht University radiation rules and regulations.

1. Pulse Chase

1. **Pulse Chase for Adherent Cells**
   NOTE: The volumes given here are based on 60 mm cell culture dishes. For 35 mm or 100 mm dishes, multiply the volumes by 1/2 or 2, respectively. This protocol uses a pulse time of 10 min and chase times of 0, 15, 30, 60, 120, and 240 min. These can be varied depending on the specific proteins being studied (discussed below).
   1. Seed adherent cells (*e.g.*, HEK 293, HeLa, CHO) in six 60 mm cell culture dishes so that they will be subconfluent (80% - 90%) on the day of the pulse chase. Use at least one dish per time point and/or condition.
   2. If required, transfect the cells with commercially available transfection reagents according to the manufacturer's instructions; or, virally transduce[8] the cells 1 day before the pulse-chase experiment with the appropriate construct for expression.
   3. Wash the dishes with 2 mL of wash buffer (Hank's balanced salt solution [HBSS]), add 2 mL of starvation medium (normal culture medium lacking methionine and cysteine and fetal bovine serum [FBS], such as minimum essential medium [MEM] without methionine and cysteine but with 10 mM HEPES, pH 7.4), and place the dishes in a 37 °C humidified incubator with 5% CO_2 for 15 min.
   4. Transfer the dishes to the racks in a prewarmed 37 °C water bath so that they are in contact with water but do not float. Start a timer.
   5. At 40 s, aspirate the starvation medium, draw up 600 µL of pulse solution (starvation media + 55 µCi/35 mm dish label, see the note preceding step 1.1.1) into the pipette, and add it gently to the center of the dish at exactly 1 min. Repeat this step at 1 min intervals for the remaining dishes. Start with the longest chase sample to save time during your experiment.
      NOTE: When handling radioactive material, it is essential to follow appropriate precautions and local rules and regulations to prevent accidental exposure and/or contamination.
   6. At exactly 11 min and for all following dishes, except for the 0 min chase sample, add 2 mL of chase medium directly to the dish, aspirate it, and again, add 2 mL of chase medium. Repeat this step at 1 min intervals for remaining dishes. Transfer all dishes to a 37 °C incubator.
   7. At exactly 16 min, add 2 mL of chase medium (normal culture medium + 10 mM HEPES [pH 7.4], 5 mM cysteine, and 5 mM methionine) directly to the 0 min sample dish on top of the pulse medium to stop labeling; then, aspirate immediately, transfer the dish to a cooled aluminum plate, and add 2 mL of ice-cold stop buffer (HBSS + 20 mM *N*-ethylmaleimide [NEM]).
      NOTE: This is the 0 min chase sample. For this sample, proceed directly to step 1.1.9.
   8. Transfer each chase dish back to the water bath 2 min before each chase time (*e.g.*, 24 min for a 15 min chase) and, at the exact chase time (*e.g.*, 26 min for a 15 min chase), aspirate the chase media (or transfer it to a microcentrifuge tube if following protein secretion) and transfer the dish to a cooled aluminum plate. Add 2 mL of ice-cold stop buffer.
   9. Incubate all dishes on ice in the stop buffer for ≥5 min; then, aspirate the stop solution and wash it with 2 mL of ice-cold stop solution. Aspirate the wash and lyse dishes with 600 µL of lysis buffer (phosphate-buffered saline [PBS] + nondenaturing detergent [see **Table of Materials**] + protease inhibitors + 20 mM NEM). Use a cell scraper to ensure that the dishes are lysed quantitatively.
   10. Transfer the lysate to a 1.5 mL microcentrifuge tube and centrifuge for 10 min at 15,000 - 20,000 x *g* and 4 °C to pellet the nuclei.

2. **Pulse Chase for Suspension Cells**
   NOTE: To ensure efficient labeling, cells should be pulsed at a concentration of 3 x 10^6 to 5 x 10^6 cells/mL, and chase volumes should be 4x the pulse volume. In the following example, we pulsed 5 x 10^6 cells in a volume of 1 mL for 10 min, to yield five chase time points (0, 15, 30, 60, and 120 min) of 1 mL, containing 1 x 10^6 cells per time point. All solutions are the same as in section 1.1.
   1. Culture the suspension cells (*e.g.*, 3T3, Jurkat) according to a previously published protocol[9] so that there is a sufficient number of cells at the time of the experiment, at least 1 x 10^6 cells per time point and/or condition.
   2. If required, transfect cells with commercially available transfection reagents according to the manufacturer's instructions, or virally transduce[8] the cells 1 day before the pulse-chase experiment with the appropriate construct for expression.
   3. Pellet 5 x 10^6 cells per condition in a 50 mL tube for 5 min at 250 x *g* at room temperature, wash them 1x with 5 mL of starvation media, pellet them again, and resuspend them in 1 mL of starvation media.
   4. Transfer the cells to a 37 °C water bath and incubate them for 10 - 25 min. Agitate the tubes every 10 - 15 min to prevent the cells from settling at the bottom.
   5. Start the timer. At exactly 1 min, add 275 µCi (55 µCi/1 x 10^6 cells) of undiluted label directly to the tube containing cells and swirl to mix.

NOTE: When handling radioactive material, it is essential to follow appropriate precautions and local rules and regulations to prevent accidental exposure and/or contamination.

6. At exactly 11 min, stop labeling by adding 4 mL of chase media. Mix the sample and immediately transfer 1 mL to a 15 mL tube on ice, containing 9 mL of ice-cold stop solution.
   NOTE: This is the 0 min chase sample.
7. Repeat these steps for each successive time point. Once all time points are collected, pellet the cells for 5 min at 250 x *g* at 4 °C. Aspirate the medium (or transfer it to a new 15 mL tube if following protein secretion). Wash the cells with 5 mL of stop solution and pellet the cells for 5 min at 250 x *g* at 4 °C.
8. Aspirate the stop solution, completely lyse the cells with 300 µL of ice-cold lysis buffer and incubate them for 20 min on ice to ensure a complete lysis. Transfer the lysate to a 1.5 mL microcentrifuge tube and centrifuge for 10 min at 15,000 - 20,000 x *g* at 4 °C to pellet the nuclei.

2. Immunoprecipitation

1. Combine antibody (see the discussion) and 50 µL of immunoprecipitation beads (e.g., protein A-Sepharose) (10% suspension [v/v] in lysis buffer + 0.25% bovine serum albumin [BSA]) in a microcentrifuge tube and incubate them at 4 °C for ~30 min in a shaker.
2. Pellet the beads for 1 min at 12,000 x *g* at room temperature and aspirate the supernatant. Add 200 µL of lysate to the antibody-bead mixture and incubate at 4 °C in a shaker for 1 h or head-over-head if the immunoprecipitation requires >1 h.
3. Pellet the beads for 1 min at 12,000 x *g* at room temperature. Aspirate the supernatant and add 1 mL of immunoprecipitation wash buffer. Place the sample in a shaker at room temperature for 5 min.
4. Pellet the beads as described in step 2.3 and repeat the wash 1x. Then, aspirate the supernatant and resuspend the beads in 20 µL of TE buffer, pH 6.8 (10 mM Tris-HCl, 1 mM EDTA). Vortex the sample.
5. Add 20 µL of 2x sample buffer without the reducing agent, vortex it, heat it for 5 min at 95 °C, and vortex again.
   NOTE: If only preparing reducing samples, 2 µL of 500 mM DTT should be added at this point. Then, proceed to step 2.7.
6. Pellet the beads as described in step 2.3. Transfer 19 µL of the nonreduced supernatant to a fresh microcentrifuge tube containing 1 µL of 500 mM DTT, centrifuge the sample, and vortex it before heating it for 5 min at 95 °C again.
7. Spin down the sample for 1 min at 12,000 x *g*; this is the reduced sample. Cool it down to room temperature and add 1.1 µL of 1 M NEM to both the reduced and the nonreduced sample. Vortex and spin down the samples.

3. SDS-PAGE

1. First, determine the appropriate SDS-PAGE resolving gel percentage for the protein of interest. For example, HIV-1 gp120, when deglycosylated, runs at ~60 kDa and is analyzed in a 7.5% gel.
2. Prepare the resolving gel mixture without TEMED according to the manufacturer's instructions (x% acrylamide, 375 mM Tris-HCl [pH 8.8,] 0.1% SDS [w/v], and 0.05% ammonium persulfate [APS] [w/v]) and degas under vacuum for >15 min. While the gel mixture is degassing, thoroughly clean the gel glass plates with 70% ethanol and lint-free tissues and place them into a casting apparatus.
3. Add TEMED to the resolving gel mixture (at a final concentration of 0.005% [v/v]), mix thoroughly, and pipette between glass plates, leaving ~1.5 cm space for the stacking gel. Carefully overlay the gel with deionized H_2O or isopropanol and leave it to polymerize.
4. Once the resolving gel has polymerized, prepare the stacking gel mixture (4% acrylamide, 125 mM Tris-HCl [pH 6.8], 0.1% SDS [w/v], and 0.025% APS [w/v]).
5. Flush the top of the resolving gel with deionized H_2O and, then, remove all water.
   NOTE: Use filter paper to remove the last drops.
6. Add TEMED to the stacking gel mixture (0.005% [v/v]), mix thoroughly, and overlay the resolving gel with stacking gel and insert a 15-well comb. Once the stacking gel has polymerized, transfer it to a running chamber and fill the upper and lower chambers with running buffer (25 mM Tris-HCl, 192 mM glycine [pH 8.3], and 0.1% SDS [w/v]).
7. Load 10 µL of sample per lane in a 15-lane minigel. Avoid loading the samples in the first and the last lane on the gel and load the nonreducing sample buffer in all empty lanes to prevent the smiling of bands. Run the gels at constant a 25 mA/gel until the dye front is at the bottom of the gel.
8. Remove the gels from the glass plates, stain the gels with protein-staining solution (10% acetic acid and 30% methanol in H_2O + 0.25% brilliant blue R250 [w/v]) for 5 min with agitation, and then, destain for 30 min with destaining solution (staining solution without brilliant blue R250).
9. Arrange the gels face-down on a plastic wrap and, then, place 0.4 mm chromatography paper on top of them. Place the gel sandwich chromatography paper-side down onto a gel dryer. Following the manufacturer's instructions, dry the gels for 2 h at 80 °C.
10. Transfer the dried gels to a cassette and overlay them with autoradiography film or phosphor screen. If using autoradiography film, this step must be performed in a dark room.

Representative Results

The folding and secretion of HIV-1 gp120 from an adherent pulse chase is shown in **Figure 2**. The nonreducing gel (Cells NR in the figure) shows the oxidative folding of gp120. Immediately after the pulse labeling of 5 min (0 min chase) gp120 appears as a diffuse band higher in the gel, and as the chase progresses, the band migrates down the gel through even more diffused folding intermediates (IT) until it accumulates in the tight band (NT) that represents natively folded gp120. This occurs as the formation of disulfide bonds increases the compactness of the protein, causing it to migrate faster than the fully reduced protein. On the reducing gel (Cells R in the figure), the disulfide bonds on all forms have been reduced such that, at all chase times, they do not affect mobility. This allows the analysis of other modifications. The shift over time from Ru to Rc represents the posttranslational signal-peptide cleavage of gp120: the mobility increases due to the loss of the signal peptide, which increases during the chase as more proteins attain the native fold and lose their signal peptide. On both the nonreducing and reducing gel, the signal begins to decrease from ~1 h onward due to the secretion of gp120. This can be monitored by analyzing the media (Medium in the figure). A comparison of the nonreducing and reducing gels uncovers disulfide bond changes and signal-peptide removal. This was only possible because another modification, N-linked glycosylation, and glycan modifications, were removed from gp120 (and the analysis) by digestion with endoglycosidase H just before SDS-PAGE.

The trafficking of the μ heavy chain of immunoglobulin M (IgM) from a suspension pulse chase is shown in **Figure 3**. A shift over time from HC_{ER} to HC_{Golgi} represents the trafficking of the μ chain from the ER to the Golgi, which precedes secretion from the cell. This change in molecular mass is caused by the modification of N-linked glycans in the Golgi.

Figure 1: Schematic diagram of the protocol for pulse chase, immunoprecipitation, and SDS-PAGE. Please click here to view a larger version of this figure.

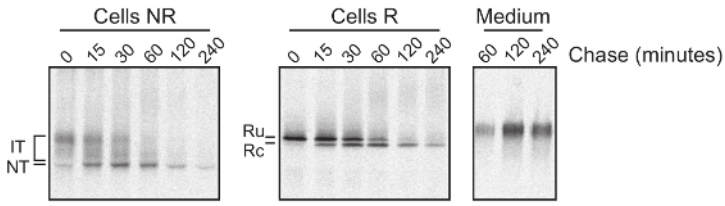

Figure 2: Folding and secretion of HIV-1 gp120, determined by adherent pulse chase. Subconfluent 60 mm dishes of HeLa cells expressing HIV-1 gp120 were pulse labeled for 10 min and chased for the indicated times. After the immunoprecipitation of gp120, the samples were deglycosylated and analyzed using a 7.5% SDS-PAGE gel. IT = folding intermediates; NT = native gp120; Ru = reduced signal-peptide-uncleaved gp120; Rc = reduced signal-peptide-cleaved gp120; NR: = nonreducing; R = reducing. Please click here to view a larger version of this figure.

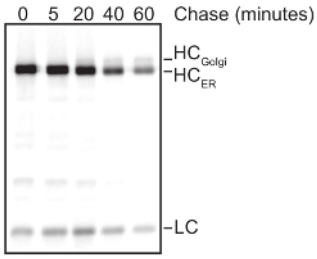

Figure 3: Trafficking of the heavy chain of IgM, determined by suspension pulse chase. 5 x 10^6 I.29 μ^+ B cells were pulse labeled for 5 min and chased for the indicated times. After immunoprecipitation, the samples were analyzed using a 10% SDS-PAGE gel. HC = immunoglobulin μ heavy chain; LC = immunoglobulin light chain. Please click here to view a larger version of this figure.

Discussion

Pulse-chase methods have been essential for developing scientists' understanding of protein folding in intact cells. While we have attempted to provide a method that is as general as possible, this approach has the potential for almost limitless variations to study various processes that occur during the folding, the transport, and the life of proteins inside the cell.

When performing a pulse chase using adherent cells in dishes, it is essential to treat each dish the same as much as possible, as a separate dish is used for each time point and/or condition in an experiment. Especially for short pulse and chase times (<5 min), it is essential to maintain a tight control over the pulse and chase times (with a digital timer). Pulse chases with suspension cells can help to reduce this variability as all samples are taken from a single tube. As some cell types adhere less well to culture dishes than others, care should be taken to ensure that the cells are not washed away during the various medium changes. This can be overcome by either using these cells in suspension or by coating the dishes with poly-L lysine or gelatin, for example, to adhere the cells to the culture dishes. To measure the reproducibility of labeling between dishes, a sample of lysate should be examined by SDS-PAGE to check all samples for identical total labeling and protein pattern. Alternatively, liquid scintillation counting of lysates will establish the total counts-per-minute (cpm) incorporated but will not provide information on the protein population labeled.

If a target protein labels poorly, the signal is increased by increasing the number of cells (or dishes) rather than by increasing the amount of label. Lengthening the pulse time is an option when the kinetics of the studied process will allow this. The ideal pulse time will vary with each target owing to factors such as the expression level, the transcription rate, the number of methionines/cysteines, and the folding rate of the protein. As such, experimentation must be undertaken to determine the best balance between the above-mentioned factors in each experiment while keeping the experimental aim in mind. If intermediates during folding are being studied, for example, it is desirable to pulse the label for as short a time as possible to have the starting material as close to an unfolded state as possible, while balancing expression levels. Alternatively, if the transport or degradation of a protein is the target of study, pulse times may be lengthened to provide the highest levels of signal possible without compromising kinetic information. In general, pulse times can range from 2 - 15 min when using this protocol without modification. Previous experimentation[5] has demonstrated a lag time of ~10 s after the pulse before the incorporation of radioactivity into the total protein pool; this is important to keep in mind when deriving kinetic information from pulse-chase experiments. For short pulse times (< 2 min), keep all the dishes on the water bath during the entire pulse time. If extended pulse times (>1 h) are being used, it is advisable to increase the pulse volume by 1.5 times and place dishes on a rocker in a 37 °C incubator during the pulse to prevent the dishes from drying out. While labeling solutions containing individual ^{35}S-methionine/cysteine or a mix are available, the mix is preferred even when proteins that only contain methionine or cysteine are being labeled as both are needed to maintain general protein synthesis in the cell. If experimental conditions require specific methionine/cysteine labeling, the levels of nonradioactive methionine/cysteine during the pulse must be adjusted accordingly.

Chase times (and the number of time points) are determined in a similar fashion. A good starting place is to set the first chase time (after the 0 min chase) as equal to the pulse time and, then, double the length of time for each successive time point (*e.g.*, 5, 10, 20, and 40 min). However, this must be optimized for each specific protein and question.

To prevent the activation of stress responses by starvation (which may affect protein synthesis and folding), ideally, some unlabeled methionine and cysteine should be added to the starvation and radiolabeling solutions. The quantity will depend on the cell line, the labeling time, the

quantity of the radiolabel used, and media volumes, and will need testing. A good starting point is 1% of the amount of cysteine and methionine present in the cell culture medium, which can be increased with increasing pulse times. Starvation periods, whether with or without unlabeled amino acids, should be kept in the range of 15 - 30 min to ensure adequate label incorporation and prevent the activation of stress responses. When using an extended pulse (≥1 h), starvation is not required.

The lysis buffer described here will be suitable for most purposes, but in principle, any buffer system, such as HEPES, Tris, or MES, will work. The detergent concentration needed to lyse cells will depend on the number of cells and detergent volume. The concentration should always be above the critical micelle concentration (CMC) and the quantity of detergent sufficient to lyse cells. An empirical rule of thumb is that 200 µL of 0.5% nondenaturing detergent (see **Table of Materials**) is sufficient to lyse the cell equivalent of ~1 mg of protein (~1 x 10^6 cells). The salt concentration and detergents may also be varied according to the application, but in general, conditions that break open nuclei (high salt, >0.1% SDS) should be avoided as the presence of free DNA will interfere with immunoprecipitation. If this cannot be avoided, for instance, when complete cell lysates including nuclei or pelleted proteins need to be analyzed, DNA can be sheared by passing the cells through a small gauge needle prior to immunoprecipitation. This is preferred over sonication so as to prevent excessive foaming and the production of radioactive aerosols.

For immunoprecipitation, both the optimal quantity of antibodies and the best wash buffer for each antibody-antigen combination must be tested thoroughly to achieve a balance between the desired antigen signal and background. Antibodies should always be present in excess over antigens to ensure quantitative immunoprecipitation; a good starting point is 1 µg of antibodies per immunoprecipitation from a 35 mm dish/1 x 10^6 cells. The background can be decreased by increasing the detergent or salt concentrations. In particular, the addition of ≤0.1% SDS can help to greatly reduce the background but may also disrupt antibody-antigen interactions. To control for the specificity of antibodies during immunoprecipitation, identical cells lacking the target protein should be used when available. If this is not straightforward, such as when endogenous proteins are being analyzed, the overexpression or depletion of the antigen will work. To control for background (and specificity), either preimmune sera (where possible) or isotype controls should be used. To control for antigens binding directly to immunoprecipitation beads, beads without antibody should also be used. The conditions provided here should be considered as a starting point as each antibody-antigen pair will require an optimization of both wash buffer and wash conditions. In case of too much background, do not increase the number of washes but rather the time of washing and/or the composition of the wash buffer. If particularly sensitive interactions such as in coimmunoprecipitations have to be dealt with, it may be preferable to carry out all wash steps at 4 °C, using ice-cold buffers.

One advantage of adherent cells over suspension cells is that drug treatments can be performed at virtually any point during the pulse chase. If used with chaperone inhibitors, for example, it is possible to differentiate its effects on the co- *versus* posttranslational phases of a folding process. This can be extended by adapting the lysis and immunoprecipitation wash buffers (discussed above) to allow the coimmunoprecipitation of protein-chaperone complexes during folding[10,11,12].

The postlysis treatment of samples will widen the scope of questions that can be addressed. The limited protease digestion of target proteins before immunoprecipitation will provide additional conformational information and has been successfully used to monitor the folding of proteins, especially those lacking disulfide bonds[13,14]. Protein aggregation and complex formation can be monitored by sucrose density-gradient centrifugation prior to immunoprecipitation[15,16].

To take full advantage of pulse chases, the availability of high-quality reagents is required for immunoprecipitations. When examining folding intermediates, it is essential to have antibodies that are capable of pulling down all forms of the protein under analysis. If specific antibodies to the target protein are not available, this can be substituted by using affinity tags. However, this severely restricts the usefulness of postlysis methods, such as the limited proteolysis, to directly probe the protein conformation, as only tagged fragments can be recovered.

The sensitivity of pulse chases is limited by the number of methionine and cysteine residues in the protein in question. Proteins with low numbers of methionine/cysteine residues coupled with a low expression level are undetectable in pulse-chase experiments. When performing postlysis modifications such as limited proteolysis, only methionine/cysteine-containing fragments will be detectable.

To conclude, given the kinetic detail that pulse chases provide, they are complementary to many other techniques and tools used to study molecular cell biology at the steady-state level. As such, they continue to be an invaluable component in the molecular biologist's toolbox.

Disclosures

The authors have nothing to disclose.

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