Published in final edited form as:

Methods Mol Biol. 2011 ; 782: 47–57. doi:10.1007/978-1-61779-273-1_5.

Checkpoints Studies Using the Budding Yeast *Saccharomyces cerevisiae*:

Analysis of changes in protein level and subcellular localization during cell cycle progression

Xiaorong Wu, Lili Liu, and Mingxia Huang

Summary

Methods are described here to monitor changes in protein level and subcellular localization during the cell cycle progression in the budding yeast *S. cerevisiae*. Cell synchronization is achieved by an α -factor mediated block-and-release protocol. Cells are collected at different time points for the first two cell cycles upon release. Cellular DNA contents are analyzed by flow cytometry. Trichloroacetic acid protein precipitates are prepared for monitoring levels of cell cycle regulated proteins by Western blotting. The dynamic changes in protein subcellular localization patterns are examined by indirect immunofluorescence microscopy.

Keywords

Cell cycle; checkpoint; cell synchronization; flow cytometry; immunofluorescence microscopy; protein stability; subcellular localization; *S. cerevisiae*

1. Introduction

Cell cycle checkpoints are regulatory pathways that ensure the proper order and timing of phase-specific events in order to maintain genomic stability [1–4]. Extensive studies from numerous laboratories have established that the basic mechanisms of cell cycle checkpoints are evolutionarily conserved in all eukaryotes [2, 5–7]. The budding yeast *Saccharomyces cerevisiae* is a popular model eukaryotic organism for the study of various cellular processes including cell cycle regulation [8]. In fact, the concept of checkpoint was initially developed in *S. cerevisiae* over two decades ago [9]. The ease of propagation and maintenance, combined with its powerful genetic, genomic, molecular, and biochemical tools, has made the budding yeast an organism of choice for studying the mechanisms underlying cell cycle checkpoints.

A critical technique in cell cycle experiments is cell synchronization. Two methods are routinely used to obtain synchronized yeast populations: centrifugal elutriation and block-and-release protocols. While elutriation can collect homogeneously dividing cells without much perturbation [10], the block-and-release methods have the advantage of easily

⁴The cell population released from α factor-mediated arrest usually proceeds through the first two cell cycles synchronously; cells will lose synchrony after the first two cell cycles.

generating much larger populations of synchronized cells for biochemical characterization of proteins of interest at different time points of the cell cycle [11, 12]. Among various block-and-release protocols the yeast pheromone α -factor is frequently used to arrest *MATa* cells at the G1/S-phase boundary (START) by inhibiting Cdc28-Cln activity [13]. The arrested cells have an 1N DNA content and a pear-shaped (“schmoo”) morphology [14]. Upon washing off α -factor in the medium, yeast cells recover quickly and progress synchronously through the first couple of cell cycles. The progression of cells through cell cycle can be monitored by examining changes in cellular morphology (budding index analysis) and by measuring cellular DNA contents using fluorescence-activated cell sorting (FACS). In this chapter, we compiled protocols of a series of time-course experiments of cell cycle analysis. Cells are synchronized in G1/S-phase boundary by α -factor mediated arrest and released back into the cell cycle, whereupon samples are collected at 10-min intervals during the first two cell cycles and processed for FACS and protein analyses.

The ribonucleotide reductase (RNR) catalyzes conversion of ribonucleoside diphosphates to deoxyribonucleoside diphosphates, a rate-limiting step in deoxyribonucleotide (dNTP) biosynthesis [15]. RNR is regulated by both the cell cycle and DNA damage checkpoints. Two regulatory processes are responsible for the increase in RNR activity and dNTP production during S phase. One event is the nucleus-to-cytoplasm redistribution of the RNR small subunit, which allows co-localization of the large and small subunits of RNR in the cytoplasm and increases holoenzyme formation [16]. Another event is phosphorylation and proteolysis of the Rnr1 inhibitor Sml1, which further enhances RNR catalytic activity [17]. In the following, we use the RNR small subunit protein Rnr2 as an example for analysis of protein subcellular localization pattern changes by using indirect immunofluorescence (IMF), and Sml1 for protein level fluctuation by using Western blotting.

2. Materials

The water for making media and solutions should be double distilled (ddH₂O) or from a Milli-Q still (Millipore). Yeast cultures are grown at 30°C.

2.1 Culture Growth and Synchronization

1. Yeast strains
MATa ade2 his3 leu2 trp1 ura3 can1
MATa ade2 his3 leu2 trp1 ura3 can1 Myc-RNR2
2. YEPD medium: 1% yeast extract, 2% peptone, and 2% dextrose.
3. α -factor is from GenScript and Sigma-Aldrich. Make 5 mg/mL stock solution in 0.1 M HCl and keep aliquots at -20°C.

2.2 Flow Cytometry

1. 70% Ethanol.
2. 1 × PBS buffer, pH 7.4: 40 mM K₂HPO₄, 10 mM KH₂PO₄, 0.15 M NaCl, pH7.4.

3. FACS buffer: 0.2 M Tris-HCl, pH 8.0, 20 mM EDTA, pH 8.0, with 0.1% RNase A to be added immediately before use.
4. 500 µg/mL × Propidium Iodide (10 × PI) stock solution: dissolve 10 mg of PI (Sigma-Aldrich) in 20 mL of 1 × PBS, pH 7.4 buffer in a 50 mL conical tube, wrap the tube in aluminum foil and store at 4°C. The 1 × PI working solution is made freshly before use by dilution of one part 10 × PI stock solution with nine parts 1 × PBS, pH 7.4.
5. Sonicator (Branson Sonifier 250).

2.3 Protein Extraction and Western Blotting

1. 20% trichloroacetic acid (TCA).
2. Glass beads, 0.5 mm size (Research Products International Co).
3. Bullet Blender™ (Next Advance Inc.).
4. 18-gauge syringe needle.
5. Sonicator (Branson Sonifier 250).
6. 1 M Tris-base (pH not adjusted).
7. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE):
 - 4 × resolving gel buffer : 1.5 M Tris-HCl, pH 8.8, 0.4% SDS;
 - 4 × stacking gel buffer 0.5 M Tris-HCl, pH 6.8, 0.4% SDS;
 - 2 × SDS protein loading buffer: 125 mM Tris-HCl, pH 6.8, 20% glycerol, 4% SDS, 2% β-mecaptomethanol, 0.01% bromophenol blue;
 - 1 × TGS (Tris-Glycine-SDS) running buffer: 25 mM Tris-base, 200 mM glycine, 0.1% SDS.
 - 10% ammonium persulfate.
 - TEMED (N,N,N,N'-tetramethyl-ethylenediamine),
 - 30–0.8% acrylamide/bis-acrylamide solution
8. 1 × Transfer Buffer: 25 mM Tris base, 195 mM glycine and 15% methanol.
9. 10 × Tris-buffered saline (10 × TBS) buffer: 200 mM Tris-HCl, pH 7.6, 1.37 M NaCl.
 - For 1 × TBST, mix 100 mL 10 × tock with 900 mL ddH₂O and add 0.5 ml Tween-20 to 0.05%.
10. Blocking buffer: 5% nonfat dry milk in 1 × TBST.
11. Antibody dilution buffer: 1 × TBST supplemented with 1% nonfat dry milk.
12. Antibodies: the polyclonal anti-Sml1 is a gift from JoAnne Stubbe lab (Massachusetts Institute of Technology, Department of Chemistry); antibodies against glucose-6-phosphate dehydrogenase (anti-G6DPH) is from Sigma-Aldrich.

Horse Radish Peroxidase (HRP) conjugated goat-anti-rabbit IgG is from Jackson ImmunoResearch.

13. Enhanced chemiluminescence reagents (Western Lightening Plus-ECL from PerkinElmer).

2.4 Indirect Immunofluorescence (IMF)

1. Formaldehyde 37% (w/v).
2. K buffer: 0.1 M KPO₄ pH6.5, 5 mM MgCl₂.
3. P buffer: 0.1 M KPO₄ pH6.5, 5 mM MgCl₂, 1.2 M sorbitol.
4. β -mecaptoethanol.
5. Zymolyase 100T (Fisher Scientific): make 10 mg/mL stock solution in 0.1 M sorbitol, aliquot and store at -20°C .
6. Poly-L-lysine (Sigma-Aldrich): make 1% stock solution with ddH₂O and store aliquots at -20°C .
7. Methanol: prechilled at -20°C in a Coplin jar.
8. Acetone: prechilled at -20°C in a Coplin jar.
9. PBS/BSA buffer: 10 mg/ml BSA in 1 $\times$ PBS, pH 7.4 with 0.01% NaN₃.
10. Antibodies: the monoclonal anti-Myc antibody 9E10 is from Covance, FITC goat-anti-mouse IgG conjugate is from Jackson ImmunoResearch.
11. 2 $\mu\text{g}/\text{mL}$ DAPI (4,6-diamidino-2-phenylindole, Sigma-Aldrich): make 1000 $\times$ stock (2 mg/mL) solution in ddH₂O, store aliquots in the dark at -20°C .
12. VECTASHIELD mounting medium (Vector Laboratories).
13. 25 $\times$ 75 $\times$ 1 mm microscope slides with hydrophobic coating, 14-well, 5 mm diameter (Thermo Scientific Erie) and 22 $\times$ 60 $\times$ 0.15 mm coverslips.
14. Rubber cement.

3. Method

3.1 Synchronization of cells by α -factor

1. Pick a single colony into 5 mL YEPD liquid to grow overnight at 30°C .
2. Dilute the overnight culture with YEPD to a cell density of $2\text{--}4 \times 10^6$ cells/mL and grow at 30°C to early-to-mid log phase ($0.6\text{--}1.0 \times 10^7$ cells/mL, see Note 1).
3. Remove a sample of the asynchronous culture before adding α -factor. Take $0.5\text{--}1 \times 10^7$ cells for FACS, $2\text{--}4 \times 10^7$ cells for IMF and $0.6\text{--}1.0 \times 10^8$ cells for protein extraction and Western blotting).

¹Cells of an overnight culture usually have reached stationary phase and need 3–4 h to resume log-phase growth. Cell density can be measured in a UV spectrophotometer at OD₆₀₀ as well as counted with a hemocytometer. An OD₆₀₀ reading of 1.0 is usually equivalent to $\sim 2 \times 10^7$ cells/mL but the correlation may vary depending on cell size.

4. Add α -factor to 5–10 $\mu\text{g/mL}$ and keep incubating at 30°C. It takes 1.5 to 3 h to arrest cells at G1/S-phase boundary at 30°C (see Note 2).
5. Begin assessing cell cycle arrest efficiency at 1.5 h after α -factor addition. Take 5 μL cell culture and examine under a microscope. Cells that were prior to START at the time of α factor addition will arrest at the G1/S-phase boundary with a “schmoo” morphology and continue to grow larger. Cells past START at α factor addition will divide (i.e. exhibiting a budded morphology) and arrest at the G1/S-phase boundary of the subsequent cell cycle. An additional dose of 5 $\mu\text{g/mL}$ α -factor may be added to the culture after the initial 1.5 h if necessary. Examine cells every half hour until they are > 95% “schmoo” (i.e. <5% budded).
6. Remove an α factor-arrested fraction for assessing synchrony (seen Notes 3–⁵). Release cells from α -factor arrest by centrifugation (4000g for 3–5 min), washing twice with prewarmed (30°C) YEPD, and resuspend in prewarmed (30°C) YEPD to a final concentration of $0.5\text{--}1 \times 10^7$ cells/mL.
7. Remove a time-zero fraction. Return the synchronized population to 30°C and begin taking time-points to collect fractions of culture every 10 min for 120–180 min.

3.2 Processing Cells for Flow Cytometry Analysis

1. Fixation. Collect $0.5\text{--}1.0 \times 10^7$ cells (see Note 5) by centrifugation and resuspend in 0.5–1.0 mL of 70% ethanol, incubate from 30 min to overnight on bench top. The cells are stable at this point for up to two weeks at 4°C.
2. Rehydration. Collect ethanol-fixed cells by centrifugation and resuspend in 1 mL of $1 \times \text{PBS}$, pH 7.4. Rehydration can take approximately 1 h if the cells have been in 70% ethanol for extended time. The cells are stable at this point for up to two weeks at 4°C. Add NaN_3 to a final concentration of 0.1% if the cells are to be kept for a while.
3. RNase A treatment. Collect rehydrated cells by centrifugation and resuspend into 100 μL of FACS buffer supplemented with 0.1% RNase A. Incubate at 37°C from 4 h to overnight.
4. PI staining. Collect cells by centrifugation and resuspend in 100 μL of $1 \times \text{PBS}$ supplemented with 50 $\mu\text{g/mL}$ of PI. Staining occurs rapidly at room temperature (within 1 h), although this is often a convenient point to leave the cells overnight at 4°C in the dark. Cells can kept for 1–2 weeks before FACS analysis.

²Cell density is critical to the success of α -factor arrest of *BAR1* strains. It is recommended that α -factor is added before the cell density reaches 1×10^7 cells/mL. In contrast, efficient α -factor arrest can be achieved at higher cell densities with *bar1* mutant strains that lack the secreted aspartyl protease and are much more sensitive to the pheromone relative to *BAR1* strains. YEPD of pH 3.9 can be used to allow more efficient arrest by α -factor, as the acidic condition inhibits the protease activity that degrades α -factor.

³For *BAR1* cells, release from arrest is quite homogenous after the washing step. Because the *bar1* mutant cells are very sensitive to residual α -factor, 0.1 mg/mL of Pronase E (Sigma-Aldrich) is used in the washing media to facilitate α -factor degradation and quick recover from the arrest.

⁵The $0.5\text{--}1.0 \times 10^7$ cells collected at each time point provide a good number of cells to track through the staining procedure and gives reasonable flow rates on the flow cytometer. For comparison of FACS profiles between samples, it is recommended that the cell density (cell number/mL) be kept fairly constant for all samples; the positions of fluorescence peaks may drift from one sample to another if there is a high variability in cell density between samples.

5. Sonication. Add 900 μL $1 \times$ PBS to each sample for a dilution of 1:10. Sonicate briefly (10–12 sec at 20% output) to break up cell aggregates before proceeding to flow cytometry.

3.3 Monitoring Protein Level Changes by Western Blotting

3.3.1 Preparing Total Protein Extracts by TCA Precipitation

1. Collect $0.6\text{--}1 \times 10^8$ cells by centrifugation and wash once with 1 mL of 20% TCA.
2. Resuspend cells in 500 μL of 20% TCA in a 1.5 ml microtube, add equal volume of 0.5 mm diameter glass beads, and disrupt cells by vortexing in the Bullet Blender at speed level 9 for 2.5 minutes at 4°C .
3. Puncture a hole at the bottom of the microtube with a 18-gauge syringe needle, insert it into another 1.5 mL tube and collect the liquid through the hole into the new tube by centrifugation at 2,000g for 30 sec.
4. Centrifuge the collected liquid at 10,000g for 10 min at 4°C .
5. Discard the supernatant, add 100 μL of $2 \times$ SDS loading buffer to the pellet and then add 50 μL of 1 M Tris base to adjust the pH, the dye will change from yellow to blue as the pH is being adjusted.
6. Sonicate each sample on the Branson Sonifier 250 for 5–10 sec at 20% output to solubilize the pellet.
7. Heat the samples at $95\text{--}100^\circ\text{C}$ for 5 min and chill on ice. Centrifuge at 2,000g for 10 min before loading.

3.3.2 SDS-PAGE and Western Blotting—We use the Bio-Rad Mini-Protean and Mini Trans-blot systems for SDS-PAGE and protein transfer to membrane for immunoblotting. For SDS-PAGE of Sml1, 10–12% gel is recommended because of the small size of the protein. Prepare 5 mL separating gel mixture (1.2 mL of $4 \times$ separating gel buffer, 2 mL of 30–0.8% acrylamide/bis-acrylamide, 1.8 mL of ddH₂O, 17 μL of 10% ammonium persulfate and 3.5 μL of TEMED) and 1 mL of stacking gel mixture (0.25 mL of $4 \times$ stacking gel buffer, 0.17 mL of 30–0.8% acrylamide/bis-acrylamide, 0.6 mL of ddH₂O, 8 μL of 10% ammonium persulfate and 1.5 μL of TEMED) for a 0.75 mm mini-gel. TCA protein suspension equivalent to $\sim 2 \times 10^6$ cells is loaded per lane. Electrophoresis conditions are 80 V for ~ 2.5 h or 20 V for 10 h, till the dye front reaches the bottom of the gel. Slower voltage and longer electrophoresis time can help better separation of different mobility species of the same protein due to phosphorylation.

Transfer of proteins to either PVDF or nitrocellulose membrane is done in $1 \times$ transfer buffer in the Mini Trans-blot tank at 300 mA for 1 h at 4°C . After transfer, the membrane is incubated first in blocking buffer for at least 1 h, then in antibody dilution buffer containing anti-Sml1 at 1:10,000 dilution for 2 h, followed by three washes with $1 \times$ TBST. The membrane is then incubated with goat anti-rabbit HRP conjugate at 1:10,000 dilution for 1 h, washed in TBST, and processed for chemiluminescence.

3.4 Immunostaining of Yeast Cells on a Microscope Slide

1. Fixing cells. Add 110 μL of 37% (w/v) formaldehyde directly into 1 mL of cell culture ($2\text{--}4 \times 10^7$ cells) in a 1.5 mL microtube so the final concentration of formaldehyde is 3.7%. Incubate the cells at 30°C for 15 min on a rolling drum.
2. Collect cells by centrifugation at 2,000g for 2 min. Wash cells first with 1 mL of K buffer and then with 1 mL of P buffer.
3. Spheroplasting cells. Resuspend cells in 1 mL of P buffer in a 1.5 mL microtube. Add 0.5 μL of β -mecaptoethanol and 5 μL of 10 mg/mL zymolyase 100T stock. Incubate in a 37°C water bath for 5–10 min (see Note 6). Start monitoring cell wall removal after 5 min of zymolyase treatment: place 5 μL of cells on a microscope slide and mount a coverslip over the cells, add 10 μL of water with a pipette from one corner of the coverslip while examining the cells under a microscope. Spheroplasts will explode in water due to osmotic shock because they lack cell wall; the burst spheroplasts also appear dimmer under the microscope comparing to intact cells.
4. Collect the spheroplasts by centrifugation at 800g for 2 min (see Note 7) and resuspend the cells in 1 mL of P buffer.
5. Preparing slides. Use one 14-well microscope slide for simultaneous processing of cell samples collected from all time points of a cell cycle time course experiment. The small well sizes (5 mm) also cut down the amount of antibodies used in IMF. Add 15 μL of 0.1% poly-L-lysine to each well and incubate at room temperature for 10 min. Aspirate off the polylysine by using a Pasteur pipett linked to a vacuum. Wash each well three times by adding a drop of ddH₂O and then aspirating it off. Let the slide air-dry after the final wash.
6. Add 15 μL of the spheroplast suspension from each time point sample to each well and wait 5–10 min for cells to settle. Aspirate the excess liquid.
7. Permeabilizing cells. Immerse the slide in prechilled methanol (–20°C) for 6 min and then in prechilled acetone (–20°C) for 20 seconds. Let the slide air-dry completely. Check cell density in each well under a microscope.
8. Blocking cells. Wash each well three times with PBS/BSA buffer.
9. Primary antibody staining. For detection of Myc-Rnr2, dilute the 9E10 monoclonal antibody 1:200 in PBS/BSA. Add 15 μL of the diluted antibody to each well. Incubate the slide in a humid chamber (e.g. a covered petri dish with a piece of wet paper towel) at room temperature for 2 h or at 4°C overnight.
10. Wash each well ten times with PBS/BSA buffer.

⁶Spheroplasting. Under the conditions described above, 5–10 minutes of zymolyase digestion is usually sufficient to achieve complete spheroplasting. Zymolyase concentration and incubation time can be adjusted to achieve optimal result.

⁷Spheroplasts are more fragile than intact yeast cells and need to be handled with more care from this step and forward. Lower centrifugation speed for spheroplast collection protects them from disintegration but may also result in some cell loss because they are lighter than intact cells. It is recommended that cell densities be checked in step 7 before proceeding to antibody staining to make sure there is a sufficient number of cells in each well.

11. Secondary antibody staining. Add 15 μ L secondary antibody diluted in PBS/BSA to each well. For goat-anti-mouse FITC conjugate, use 1:1,000 dilution.
12. Incubate the slide in a dark and humid chamber at room temperature for 2 h.
13. DNA staining. Add 15 μ L of 2 μ g/mL DAPI solution to each well and leave 3 min.
14. Wash each well ten times with PBS/BSA, aspirate the excess liquid after each wash.
15. Add 3 μ L of VECTASHIELD mounting medium to each well, and mount a coverslip onto the slide. Seal the coverslip with rubber cement.
16. Examine the cells immediately with a fluorescence microscope. The cells can be kept at -20°C in the dark for up to 1 week.

References

1. Murray A. Cell cycle checkpoints. *Curr Opin Cell Biol.* 1994; 6(6):872–876. [PubMed: 7880536]
2. Elledge SJ. Cell cycle checkpoints: preventing an identity crisis. *Science.* 1996; 274(5293):1664–1672. [PubMed: 8939848]
3. Hartwell L, et al. Cell cycle checkpoints, genomic integrity, and cancer. *Cold Spring Harb Symp Quant Biol.* 1994; 59:259–263. [PubMed: 7587077]
4. Kastan MB, Bartek J, et al. Cell-cycle checkpoints and cancer. *Nature.* 2004; 432(7015):316–323. [PubMed: 15549093]
5. Sclafani RA, Holzen TM. Cell cycle regulation of DNA replication. *Annu Rev Genet.* 2007; 41:237–280. [PubMed: 17630848]
6. O'Connell MJ, Walworth NC, Carr AM. The G2-phase DNA-damage checkpoint. *Trends Cell Biol.* 2000; 10(7):296–303. [PubMed: 10856933]
7. Gardner RD, Burke DJ. The spindle checkpoint: two transitions, two pathways. *Trends Cell Biol.* 2000; 10(4):154–158. [PubMed: 10740270]
8. Forsburg SL. The art and design of genetic screens: yeast. *Nat Rev Genet.* 2001; 2(9):659–668. [PubMed: 11533715]
9. Hartwell LH, Weinert TA. Checkpoints: controls that ensure the order of cell cycle events. *Science.* 1989; 246(4930):629–634. [PubMed: 2683079]
10. Johnston LH, Johnson AL. Elutriation of budding yeast. *Methods Enzymol.* 1997; 283:342–350. [PubMed: 9251032]
11. Walker GM. Synchronization of yeast cell populations. *Methods Cell Sci.* 1999; 21(2–3):87–93. [PubMed: 10728641]
12. Breeden LL. Alpha-factor synchronization of budding yeast. *Methods Enzymol.* 1997; 283:332–341. [PubMed: 9251031]
13. Dirick L, Bohm T, Nasmyth K. Roles and regulation of Cln-Cdc28 kinases at the start of the cell cycle of *Saccharomyces cerevisiae*. *EMBO J.* 1995; 14(19):4803–4813. [PubMed: 7588610]
14. Perlman R, et al. Rapid intracellular alkalization of *Saccharomyces cerevisiae* MATa cells in response to alpha-factor requires the CDC25 gene product. *Cell Signal.* 1989; 1(6):577–586. [PubMed: 2561949]
15. Jordan A, Reichard P. Ribonucleotide reductases. *Annu Rev Biochem.* 1988; 67:71–98. [PubMed: 9759483]
16. Yao R, et al. Subcellular localization of yeast ribonucleotide reductase regulated by the DNA replication and damage checkpoint pathways. *Proc Natl Acad Sci U S A.* 2003; 100(11):6628–6633. [PubMed: 12732713]
17. Zhao X, et al. The ribonucleotide reductase inhibitor Sml1 is a new target of the Mec1/Rad53 kinase cascade during growth and in response to DNA damage. *EMBO J.* 2001; 20(13):3544–3553. [PubMed: 11432841]

Fig 1.

Change in the Sml1 protein levels throughout the cell cycle. (A) FACS profiles of cells collected at 10-min intervals after release from α -factor mediated arrest at the G1/S-boundary. (B) Western blotting of the endogenous Sml1 protein in cell extracts from the different time points in (A). Sml1 levels is lower in the first and second S phases (during the 10–30 min and 80–90 min time point periods, respectively) and peak in the two G2/M phases (50–60 min and 110–120 min, respectively). The G6PDH enzyme was probed on the same blot as a loading control.

Fig 2.

Change of Rnr2 subcellular localization patterns at different phases of the cell cycle. Cells harboring N-terminally Myc-tagged *RNR2* that was integrated in its own chromosomal locus were synchronized by α -factor mediated arrest-and-release. Samples were taken from the G1/S-boundry (0 min), the first S phase (30 min) and the first G2 phase (60 min) for IMF analysis. FITC images show signals from Myc-Rnr2; DAPI images show signals from nuclear and mitochondrial DNA. FACS profiles of cells are shown on the right. Rnr2 is predominantly localized in the nucleus in cells from the G1 and G2 phases, but become ubiquitously localized between the nucleus and the cytoplasm in cells from the S phase.