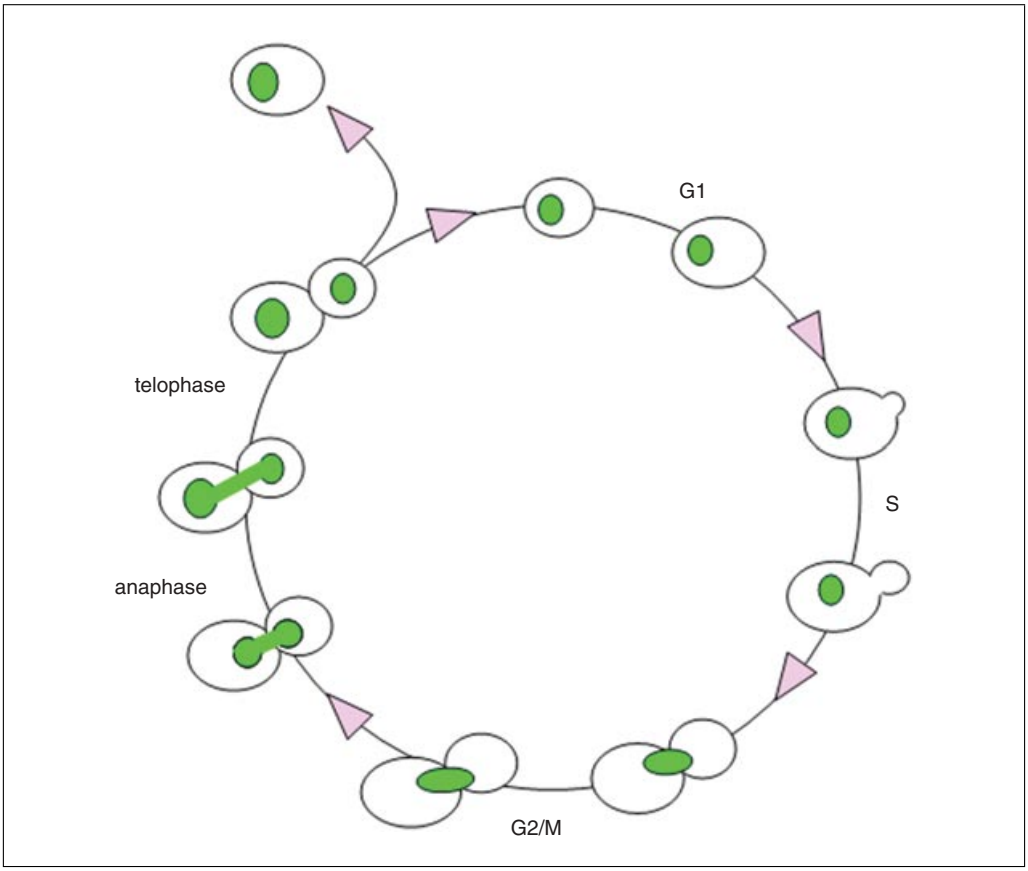

Figure 9.32.2 Pictorial representation of cellular and nuclear morphology of *S.cerevisiae* during cell cycle progression.

experiment, wild-type cells should be collected under normal growth conditions and these cells should be used to create the cell-cycle analysis template. This template can then be modified as needed for subsequent experiments. The preparation of fixed cells and the procedure for collecting data described in this unit should also be suitable for any type of subsequent morphological analyses. An example of the type of morphological analyses that can be performed with ImageStream data from budding yeast is described in Support Protocol 5.

PREPARATION OF YEAST CELLS FOR CELL CYCLE ANALYSIS

BASIC PROTOCOL

This protocol includes the culture and fixation techniques suitable for preparing samples for combined DNA content measurements and bright-field image analysis. These culture techniques can be adjusted to strain-specific requirements or different experimental growth conditions as necessary. Once the cells have been collected and fixed, they can be stored indefinitely as long as conditions are sterile.

Materials

Yeast cells and appropriate medium
70% ethanol/30% sorbitol
50 mM sodium citrate (diluted from 0.5 M stock and filter sterilized)
50 mM sodium citrate containing 0.5 mg/ml RNase A, prepare fresh
Pepsin solution (see recipe), prepare fresh
50 mM SYTOX Green stock solution in DMSO (excitation maxima: 504 nm, emission maxima: 532 nm; Invitrogen, cat. no. S7020)