expression profiles of mutants with overexpression of *HXK2* and *HAP4* showed significant overlap with CR-treated cells. A similar protocol was used in later studies to show the importance of the TOR pathway and transcription factors Msn2 and Msn4 in mediating life span extension by CR. In addition, many genes and factors were identified in yeast as important regulators of life span. The life-extending properties of many drugs, remedies and natural extracts were later discovered using this method.[21–23]

Chronological life span is also described for unicellular organisms such as yeast *Saccharomyces cerevisiae*. Determination of chronological life span requires a measurement of the number of alive cells able to produce colonies while transferred to fresh media over different time points.[24] Cells are quiescent in the G_0 phase of the cell cycle but are metabolically active thus having some similarities to those of postmitotic tissues in adult multicellular organisms. Taking into account the similarities of basic biochemical pathways in yeast and other organisms, the model of yeast replicative and chronological life spans can be successfully used for studies involving the effects of metabolism on the life span of postmitotic cells.[25,26] A few pathways mediate chronological life span and act as mediators of its extension under CR. Well-known yeast signaling pathways, namely Tor/Sch9 and Ras/AC/PKA, were shown to be sensitive to nutrient availability and activate transcription factors Msn2, Msn4, and Gis1, which are responsible for metabolic reprogramming during starvation *via* protein kinase Rim15. The Tor/Sch9 pathway also regulates the respiration and membrane potential of mitochondria. CR inhibits this pathway to decrease ROS production and increase the stress response of the cell. However, many intermediate components of the aforementioned signaling pathways are still missing to link CR effects on apoptosis, protein aggregation, genome stability and epigenetic machinery of gene regulation.[27–30]

10.2.2 CR in Worms: *Caenorhabditis elegans*

C. elegans round worms are extensively used in aging research. The standard diet for worms consists of attenuated *E. coli* bacteria placed on solid plates. There are several methods to induce CR in these organisms: dilution of bacteria in liquid cultures; dilution of peptone, which reduces bacterial growth; using axenic medium or chemically defined liquid medium; serial bacterial dilution or total absence of bacteria in plates. Klass observed life span extension by bacterial restriction for the first time in 1977 (Figure 10.1). He did a 10-fold dilution of the initial bacterial culture and in these conditions worms lived about 52% longer.[31] A similar protocol was used in other studies that showed longer life spans in animals fed diluted bacterial culture.[32–34] Removal of peptone from plates with nematode growth medium (NGM) agar extended the life span of worms by 30% but reduced reproduction.[35] Significant extension of worm life span was observed on axenic medium with killed *E. coli*, soy protein, yeast extract and hemoglobin.[36] Similarly, a longer life span was observed for worms fed chemically defined *C. elegans* maintenance