Various hormetins that have been reported to modulate aging and longevity in cells and model organisms include heat shock, irradiation, heavy metals, pro-oxidants, acetaldehyde, alcohols, hypergravity, exercise, mechanical stretching, electromagnetic field, food restriction and mental challenge.[39,51,52] Nutritional hormetins, especially those derived from plant sources, have generated much scientific interest for their health beneficial effects. This is because of the realization that not all chemicals found in plants are beneficial in a simple and straight-forward manner. Instead, these non-nutritional food components cause molecular damage by virtue of their electrochemical properties and have a typical biphasic hormetic dose response. Some examples of nutritional hormetins are those containing phenolic acids, polyphenols, flavanoids, ferulic acid, geranylgeranyl, rosmarinic acid, resveratrol, kinetin, zinc, and the extracts of tea, dark chocolate, saffron and spinach.[5,53] Chronic CR, intermittent fasting and CR-mimetics, including rapamycin and its analogues, are other examples of nutritional hormetins as drugs for healthy aging and longevity.[54–56]

7.5 Discovering Novel Hormetins

Putting test materials through a screening process for their ability to induce one or more SR pathways in cells and organisms is a promising strategy for discovering novel hormetins.[5] A general scheme for screening natural and synthetic single compounds or complex extracts as hormetins for human beings involves initial testing by using normal diploid human cells in culture. The use of normal diploid cells is very important for such studies, since immortal cell lines usually have one or more genetic and metabolic deviations, which are rarely comparable to normal cells. An important aspect of normal diploid cells is the Hayflick phenomenon of limited proliferative capacity and replicative senescence, which is a model of aging *in vitro*.[57]

Determining dose-dependent, time-dependent and age-dependent SR profiles is the first step in discovering novel hormetins.[13,40,41] Since most of the early SR markers are transcription factors (see Table 7.2), which undergo post-translational modifications and translocate from the cytoplasm to the nucleus, immunofluorescence microscopy showing this cytoplasm-to-nuclear shift may be sufficient at this stage. However, for identifying the late SR effectors, such as induced synthesis of Hsp, chaperones, cytokines, sirtuins and other antioxidative enzymes, both gene-array expression analysis for mRNA levels and proteomic analysis for protein levels will be required.

The initial screening of test materials as potential hormetins by determining their effects on early and late SR markers must be followed by performing cell type-specific functional assays. Furthermore, the cell type to be used for such a screening will depend on the biological end-point that one expects to improve by hormetin treatment. Some of the cell type-specific assays that can be used for testing novel hormetins are: cellular motility and wound healing assay for fibroblasts, induction of differentiation for stem cells and keratinocytes, blood vessel formation by endothelial cells, osteocalcin and mineralized matrix formation by osteoblasts, and muscle fiber formation by