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PERSPECTIVE

TRANSCRIPTOMICS OF CRYOPRESERVED CELLS

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Abstract

Cryopreservation is a well-known strategy to conserve genetic resources at ultra-low temperature. However, there is still limited knowledge on the cellular processes and molecular adjustments that allow cells to withstand the multiple stresses to which they are exposed during cryopreservation. To evaluate these processes, transcriptomics, the sub-discipline of omics that simultaneously examines mRNA transcripts formed by transcription from the genome, has been recently used. This article reviews recent scientific studies which use the basic principles of cryopreservation practices together with transcriptomics approaches, within the conceptual framework of cryobiomics. Moreover, the connections between factors that may be useful to optimize and validate approaches for mammalian or plant cell cryopreservation are also assessed. Transcriptomic applications are mainly performed with methods such as reverse transcriptase polymerase chain reaction (RT-PCR), simultaneous polymerase chain reaction (real-time PCR), northern blot, microarray/biochip and gene expression analysis (SAGE). Transcriptomic technologies allow a global view of gene expression profiles of different mammalian or plant cell types to be obtained before and after cryopreservation under multiple stress conditions. For these processes, small amounts of RNA enable efficient transcriptomics analysis. Transcriptomic analysis of cryopreserved mammalian and plant cells provides a conceptual way to identify the genes and their relative alterations in transcriptional abundances together with non-coding RNAs involved in important pathways related to cell viability and proliferation during and after cryopreservation. Moreover, it greatly contributes to understanding of non-fatal cryodamage and related developmental disorders in cryopreserved mammalian oocytes and sperm. In addition, single cell transcriptomics has the potential to non-invasely monitor immune actions and to diagnose the stage of the inflammatory process in kidney. Finally, qRT-PCR and RNA-seq studies have also revealed that some transcription factors are effective at inducing cold tolerance in many plants by elevating the levels of soluble sugars, proline and unsaturated fatty acids in cells. Hence transcriptomics studies may also aid investigations of the main mechanisms behind the so-called ‘cryo-recalcitrance’ that is observed mostly in plant cells.

Keywords: cryopreservation; microarray; omics; real-time PCR; reverse-transcriptase PCR.

INTRODUCTION

The term “omics” was first introduced from the word genome by Hans Winkler in the 1920s. With the advances in molecular biology studies, this term started to be used widely in the 1990s. In the 2000s, omics technologies spread to many study areas such as transcriptomics, proteomics, and metabolomics (1, 2). These branches of science are concerned with the examination of biological formations such as the genome, proteome, transcriptome and metabolome (3, 4).

It is known that systems biology, which can be defined as the systematic study of complex interactions in biological systems, uses all high-throughput omics analyses together with bioinformatics (Fig. 1) to store information of a given biological system (5, 6). In this context, all omics technologies are interconnected and follow a pattern. For instance, transcriptomics examine the mRNA produced by DNA transcription in a cell, tissue, or organism over a certain period of time, while proteomics study the structure and functions of the total proteins (proteome) synthesized by the genome in a certain tissue. On the other hand, metabolomics focus on the profiles of small molecules (metabolites) that are the products of biochemical processes in cells (7, 8).

Unlike the genome, which is roughly fixed for a cell line, the transcriptome can change with environmental factors such as nutrient types, temperature and pH changes, abiotic and biotic stresses and signals from other cells. As different functions are performed within the cell, the transcription of genes associated with these functions increases, and the transcriptome covers all mRNAs in the cell, reflecting the genes that are active at a given time. Revealing the changes in gene expression according to environmental factors is an indispensable part of systems biology in terms of environment-system interactions (9, 10). These mRNAs could be analyzed with single gene analysis such as Northern Blot, Reverse Transcription Polymerase Chain Reaction (RT-PCR) and genome wide analysis including microarray, RNA sequencing and serial analysis of gene expression (SAGE) (Fig. 1) (11).

Following RNA isolation from the cell or tissue, RNA is run in gel electrophoresis and then transferred to a suitable membrane for hybridization in Northern Blot. The advantage of this method is that it can be used to determine the size of the RNA copy of the gene and to investigate the possibility of different RNA products in different (mammalian or plant) tissues. However, quantification may not be sensitive using this technique with low transaction volumes (12, 13). To overcome this problem, isolated RNA mostly converted to cDNA using reverse transcriptase enzymes, and then c-DNA is amplified by PCR using RT-PCR. Real-time PCR can also be used instead of classical PCR for amplifying cDNA since it allows simultaneous measurement of the amount of DNA amplified in the reaction tube. A weakness of the RT-PCR technique is that an exact copy of the entire RNA molecule cannot often be obtained since the primer binding sites are generally not at the very end of the transcript. However, the sensitivity of the method is higher than Northern Blot and lower than microchips (14, 15).

Microchips enable the rapid study of single nucleotide polymorphism (SNP) or modified gene expression (increases and decreases in mRNA) in different physiological conditions. With the microchip technology, it is possible to examine more than one DNA region (almost the whole genome) on a glass chip (array) at a time with high sensitivity. DNA microchips can also be used to detect RNA that may or may not be translated into active proteins (16, 17).

The expression levels of transcripts can also be determined using SAGE, in which oligonucleotide tags (approximately 10-base long) are used for each mRNA expressing a gene in a sample and, after a series of processes, each product is evaluated by cloning and sequencing. Thus, each labeled product corresponds to a mRNA, and the number of labels gives the amount of mRNA. This method can be applied to genes whose sequences are known and it is important that the labels do not resemble each other. It is used to identify differentially expressed genes based on the comparison of gene expression data obtained from two different sources (18).

With the advent of next-generation sequencing, it has become possible to develop more accurate platforms to probe the genome and transcriptome dynamics (19). RNA sequencing (RNA-seq)-mediated transcriptomic profiling allows analysis of the expression of different genes in a sample with bulk sequencing (assuming cells of a particular type are identical) and to estimate an average of expressions.

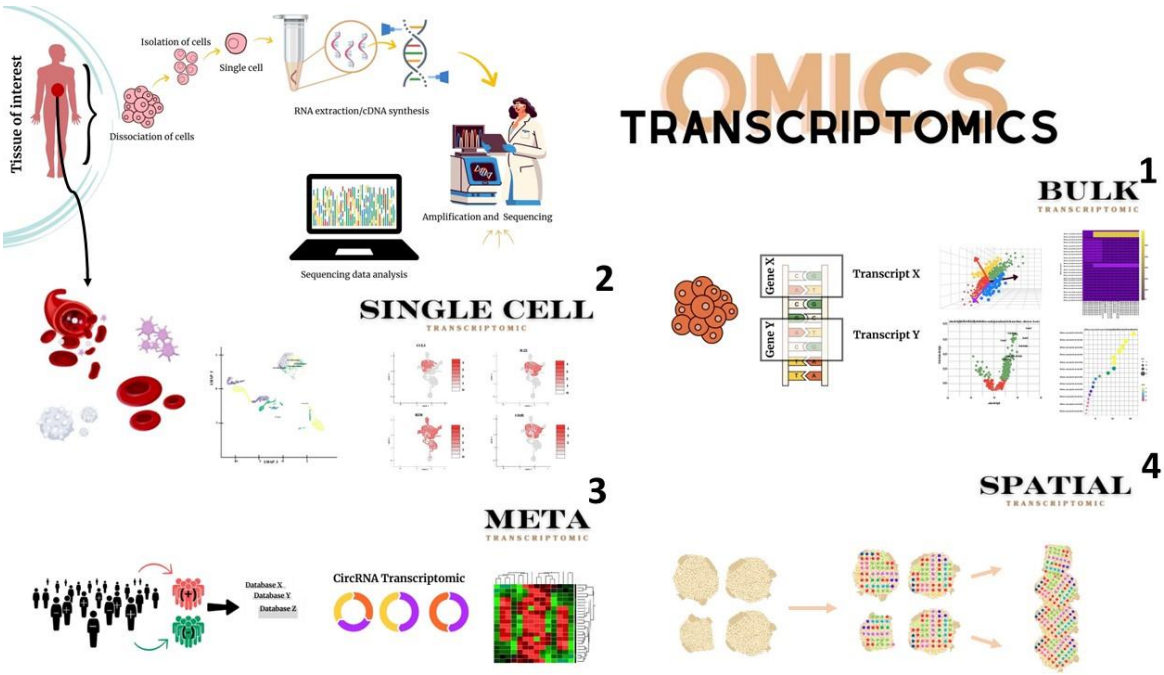

Figure 1. Different methods used in transciptomics.

However, all of these above-mentioned techniques determine the expression level in a bulk RNA that represents a population of cells. Therefore, single-cell transcriptomics analysis has been developed recently to allow single-cell sequencing (20). This technique enables researchers to quantify minute transcript amounts from up to thousands of single cells, although it requires immediate sample processing which necessicitates complex study setups (21).

All of the above-mentioned transcriptomics techniques could pave the way to understand the cellular processes and molecular adjustments that provide tolerance to the multiple stresses to which the cells are exposed during cryopreservation. Cryopreservation, which is based on the ability to preserve living cells and tissues in a quiescent status at ultra-low temperature (usually -196 °C), is used for long-term germplasm conservation of plant and/or animal cells and tissues. For cryopreservation applications, it is important to verify that there is no genetic instability in the cells or tissues to be cryo-stored before and/or after freezing and that the applied method will not have negative effects on regeneration after thawing (22, 23). Thus, in order to better understand the possible genetic expression alterations during and after cryopreservation, recent transcriptomics studies have been integrated into cryostudies (24, 25, 26).

Cryobionomics approaches focus on the connections between possible pre- and post-cryo damage and genetic stability as well as the regeneration behavior and functionality of cells and tissues in post-cryostorage recovery. Moreover, it investigates factors that may be related to possible cell/tissue injuries and to viability losses in cryopreservation treatments with possible risks of genetic instability (27, 28). In this context, the aim of this article is to review recent studies in the literature that use the basic principles of cryopreservation practices together with transcriptomics approaches within the conceptual framework of cryobiomics. Moreover, the connections between factors that may be useful in the optimization and validation of approaches for the preservation of cryostored mammalian or plant cells are also assessed.

TRANSCRIPTOMICS OF CRYOPRESERVED MAMMALIAN CELLS AND TISSUES

In general, cell cryopreservation procedures include the following successive steps: cryoprotectant solution treatment, slow cooling to below the freezing temperature to prevent lethal ice formation, thawing after cryostorage,

and removal of cryoprotectant solution (29, 30). Cryodamage that may occur in cells can be due to the cytotoxicity of the cryoprotective solution, osmotic damage caused by the cryoprotective solution during the freezing and/or thawing stages, and to intracellular ice crystal formation during freezing and/or thawing (31, 32).

To develop methodologies for effective cryopreservation of cells, it is important to understand the biological processes and pathways affected by cells during the freezing and thawing steps (33, 34). Mammalian cells are often cryopreserved in a cryoprotectant solution containing dimethyl sulfoxide (Me_2SO_4), followed by slow cooling and then they are plunged into liquid nitrogen. Me_2SO_4 is widely used in many cryopreservation protocols at concentrations ranging from 5 to 20%. Me_2SO_4 is not only effective in maintaining the internal and external osmotic balance in cells, but it also prevents ice crystal formation, which can be fatal during freezing at ultra-low temperature (35, 36).

Cell lysis and lethal ice nucleation, which can be seen in cryopreserved cells, are easily detected cryodamages in cells. However, possible DNA damage, which is also a type of cryodamage that appears over time – such as changes in gene expression or protein function – may not be fatal. For example, most cryopreserved mammalian oocytes appeared morphologically normal after thawing but failed to be fertilized or exhibited developmental problems after fertilization (37). It is known that gene expression analyzes will make great contributions to understanding by elucidating such non-fatal cryodamage and related developmental disorders. Indeed, cryodamage on the entire transcriptome or some genes in oocyte cells after cryopreservation have been

Table 1. Transcriptomics studies reported in cryopreserved mammalian cells and tissues.

Organism	Cell / tissue	Gene	Gene function	Method of analysis	Reference
Bovine	Oocyte	*KIF$_2$C* and *KIF$_3$A*	Chromosomal structure maintenance genes that can suppress oocyte development	Smart-seq2 and qRT-PCR	(38)
Bovine	Oocyte	*CHEK$_2$* and *CDKN$_1$B*	Cell cycle regulator	Smart-seq2 and qRT-PCR	(38)
Bovine	Oocyte	*Fas*, *FasL*, *Bax* and *Bcl-2*	Apoptotic genes	qRT-PCR	(39)
Human	Oocyte	*CLTA*, *CKS2*, and *MAPK6*	Cell cycle regulation and processes	qRT-PCR	(40)
Human	Oocyte	*NAP1L1*, *H1F0H1*, and *TOP1*	DNA structural organization	qRT-PCR	(40)
Human	Oocyte	*DPPA3*, *FOXJ2*, and *OCT4*	Staminal cell potency-development competence stage	qRT-PCR	(40)
Human	Oocyte	*SDHC* and *ATP5GJ*	Mitochondrial energetic pathways	qRT-PCR	(40)
Human	Oocyte	*REC8*, *SMC*, *RAD21*, *SCC3*, *SMC1A*, *STAG3*, and *SMC1B*	Chromosomal structure maintenance	qRT-PCR	(40)
Human	Cardiac and hepatic microtissues (MTs)	*DICER1, AGO2*	Cleaves pre-miRNAs, encoding the main component of the miRNA-RISC	Next Generation Sequencing	(41)

reported (Table 1). For example, significant changes as a result of cryopreservation have been determined in the mRNA levels of many genes including *KIF$_2$C* and *KIF$_3$A* (chromosomal structure maintenance genes that can suppress oocyte development) and *CHEK$_2$* and *CDKN$_1$B* (cell cycle regulator) (38), *Fas*, *FasL*, *Bax* and *Bcl-2* (apoptotic genes) (39), and *CLTA*, *CKS2*, and *MAPK6* (cell cycle regulation and processes), *NAP1L1*, *H1F0H1*, and *TOP1* (DNA structural organization), *DPPA3*, *FOXJ2*, and *OCT4* (staminal cell potency-development competence stage), *SDHC* and *ATP5GJ* (mitochondrial energetic pathways), *REC8*, *SMC*, *RAD21*, *SCC3*, *SMC1A*, *STAG3*, and *SMC1B* (chromosomal structure maintenance) (40, 41).

Similarly, cryopreserved sperm used in artificial insemination in cattle had lower fertilization quality than fresh sperm, which demonstrates that freezing changes sperm phenotypic properties and causes cryo-injury (42). Currently, the various omics technologies, including transcriptomics and proteomics, have been used in sperm cryobiology not only for exploring the molecular alterations caused by cryopreservation but also for identifying specific proteins that could be added to semen diluents prior to cryopreservation to improve sperm cryo-survival (43). Moreover, investigations on the effect of possible cryodamage on cells at the transcriptomic level in bull spermatozoa cells revealed the up-regulation of 241 genes, the down-regulation of 662 genes, and the neutral expression of 215 genes in cryopreserved spermatozoa cells in comparison with fresh spermatozoa cells (44). These findings suggest that the freezing process increases the number of sperm transcripts associated with potential fertility-related functions and pathways, which may be the cause of reduced fertility in cryopreserved bull spermatozoa (44). Moreover, differentially expressed genes in cryopreserved and vitrified spermatozoa have been evaluated by high-throughput RNA-seq in human fresh (control), frozen, and vitrified spermatozoa cells (45) since cryopreservation of spermatozoa cells is important in maintaining fertility options for male individuals. As a result, 1103 differentially expressed genes were observed in frozen spermatozoa and 333 genes in vitrified spermatozoa. The alterations of expression in these genes suggested that freezing induced more down-regulation of apoptosis and immune response related genes. Moreover, this study confirmed that cryogenic storage of human spermatozoa is an epigenetically safe method for maintaining male fertility.

The single-cell transcriptomic data generated from kidney biopsy samples from lupus nephritis patients enabled the characterization the cell populations [infiltrating and resident immune cells together with parenchymal cells (46)] related with the disease. Moreover, kidney transcriptomics provided new molecular biomarkers associated with the inflammatory process induced by the deposition of circulating immune complexes in the kidney diseases, which could help to diagnose the stage of the inflammatory process (47). These studies also demonstrated the analysis and gene expression potential of cryopreserved cells with the help of multiplexed and complementary high-dimensional analyses.

TRANSCRIPTOMICS OF CRYOPRESERVED PLANT CELLS AND TISSUES

When plant cells and tissues are exposed to sudden changes in environmental conditions, they undergo a cold acclimation process with a number of arrangements at the molecular level in order to maintain their physiological and metabolic intracellular balance, including the accumulation of intracellular and/or intercellular molecules such as osmolytes and/or cryoprotectants such as soluble sugars, which can also alter cellular membrane composition (48, 49). With the conformational changes in membrane stability and proteins as a result of the transition of the plasma membrane from the liquid crystal state to the solid gel state, Ca^{+2} ions, which are secondary messengers, are released from the cell surface through Ca^{+2} channels, and from within the cell from some organelles (such as the endoplasmic reticulum and mitochondria) into the cytosol, causing the signal to be transferred into the cell. This causes the activation of various signal transduction pathways by triggering protein kinases and a number of transcription factor cascades. Thus, the activation and/or inhibition of proteins such as cold-induced heat-shock proteins (Hsps), late embryogenesis abundant (LEA) proteins, cold-regulated (COR) proteins, inducer of calmodulin-binding expression (ICE) proteins are achieved, and eventually a response to stress

Table 2. Transcriptomics studies reported in cryopreserved plant cells and tissues.

Species	Cell / tissue	Gene	Gene function	Method of analysis	Reference
Mentha x piperita	Meristems	*mac_4* (HSP80-like)	Cold stress regulation	qRT-PCR	(53, 63)
Arabidopsis thaliana	Seedlings obtained from mature seeds	*NnRab18*	Cytoprotection	qRT-PCR	(64)
Medicago sativa	Whole plant	*MsDREB*	Specifically bind to the DRE sequence and activate the expression of genes	qRT-PCR	(65)
Solanum tuberosum cv. *Superior*	The leaves of in vitro grown plants	*CBF1*	Bind to DRE/CRT cis-element and regulate the expression of stress-responsive genes	qRT-PCR	(66)
A. thaliana and *Oryza sativa*	Seedlings obtained from mature seeds	*OsMYB3R- 2*	Transcription factor involved in abiotic stress regulation	qRT-PCR and Microarray	(60)
Pyrus betulaefolia	Leaves from 45-day-old seedlings	*PbrMYB5*	Activator of AsA (ascorbic acid) biosynthesis	qRT-PCR	(59)
A. thaliana	Whole plant	*CAMTA*	Calcium signaling	qRT-PCR	(61)
Oryza rufipogon Griff.	Seeds	*LTG5*	Regulating growth and development	RNA-Seq and qRT-PCR	(67)
A. thaliana	Seedlings obtained from seeds	*MeTCP4*	Leaf development, flower symmetry, shoot branching, and senescence	RNA-Seq and qRT-PCR	(68)
Vitis vinifera L.	Tissue culture derived-seedlings	*VaDof*	Mediates both DNA–protein and protein–protein interactions	RNA-Seq	(69)

occurs at the molecular level within the nucleus (Fig. 2, Table 2) (26, 50, 51, 52, 53).

One of the most important environmental factors affecting the viability, growth, and development of plant cells after cryopreservation is cold stress (54). Plant cells create a response that provides tolerance to this stress by regulating the expression levels of a number of genes and proteins with different functions. These responses, which have a direct or indirect role in plant cells, include cryoprotectant peptides, chaperones, transcription factors and kinases (26, 52, 53).

As transcription factors, C-repeat/dehydration responsive element-binding factors (CBFs), also known as dehydration-sensitive element-binding factors (DREBs), are members of the CBF/DREB APETALA2/Ethylene-Sensitive transcription factor (AP2/ERF) superfamily and play a crucial role in the expression of cold-induced COR genes. They positively control the expression of the relevant genes by binding to special sequences containing highly conserved CCGAC bases, also known as DRE/CRT, in the promoter region of cold and dehydration-sensitive genes, and thus ensure the synthesis of proteins responsible for the cold stress response. It has been reported in the literature that proteins synthesized through CBF/DREB transcription

factors in many plants are effective in the formation of cold tolerance by inducing the increase of soluble sugars, proline accumulation, and unsaturated fatty acids (52, 55, 56, 57).

Another transcription factor that increases expression in cells during the cold response is the myeloblastosis (MYB) family which constitutes almost 9% of the total transcription factors in plants. These proteins are basically divided into four subgroups according to the domain consisting of different repeats at their N-terminal ends. It has been reported in various studies that they positively regulate the expression of many proteins involved in the response to abiotic stress (58, 59). Using transgenic technologies, overexpression of these transcription factors in *Arabidopsis thaliana* and *Oryza sativa* plants has been shown to be effective in gaining tolerance to freezing stress in both species. In another study, it was shown that binding a MYB gene as a transcriptional activator in *Pyrus betulifolia* positively affected the synthesis of acetylsalicylic acid, which is effective in cold stress tolerance (60, 61).

Transcription factors belonging to the calmodulin-binding transcription activator (CAMTA) family have a domain for calmodulin binding. The N-terminal domains of these proteins contain a domain that provides specific binding to DNA. This plays an active role in the cold stress response and positively affects the expression of this transcription factor (CBF2), allowing the plant to gain cold tolerance (61). The expression levels of heat shock proteins (Hsps), also known as molecular chaperones, during stress are regulated by transcription factors called heat shock factors (Hsfs). Hsp molecules, whose expression level changes depending on temperature, contribute to stress tolerance by carrying out events such as protein folding, degradation, and localization within the cell (62).

Plant cells can also, in cases of freezing, experience excessive production of reactive oxygen species (ROS) due to cold stress, which disrupts the electron transport chain and is toxic to cellular processes. To overcome this negative effect, cells have developed mechanisms to eliminate ROS, including the production of enzymatic and non-enzymatic antioxidants. These enzymatic antioxidants include peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) (75)

GENE EXPRESSION ANALYSIS OF NON-CODING RNAs IN CRYOPRESERVED CELLS AND TISSUES

Non-coding small RNAs (miRNAs)

miRNAs are naturally occurring, small, single-stranded non-coding RNAs that control gene expression at the post-transcriptional and translational levels in both plant and animal cells (70, 71). miRNAs are transcribed from endogenous genes as large RNAs that have undergone post-transcriptional modification (having a CAP structure and being polyadenylated). RNA polymerase II is involved in transcription to generate large pre-miRNA transcripts in the nucleus (72).

Transcriptional and translational control greatly benefits the cell in responding to various stresses, such as cold stress. The main function of miRNAs is to repress gene expression at the translational level by binding to mRNAs. Although the exact function of many of the newly discovered miRNAs has only just been revealed, their ability to regulate cell proliferation and cell death has been reported by recent studies (73).

In a study by Xi et al. (74), miRNA expression profiles between formalin-fixed paraffin-embedded (FFPE) samples and fresh frozen samples were systematically investigated using miRNA array analysis. According to the study results, different formalin fixation times did not change the stability of miRNAs according to qRT-PCR analysis. No significant differences were observed when miRNA expressions were compared among 40 different colorectal cancer FFPE samples. This study lays a foundation for miRNA research using FFPE samples in cancer and other types of diseases.

Similarly, in another study by Weng et al. (76), whole-genome smRNA sequencing was performed on paired frozen and FFPE samples of benign kidney and renal cell carcinoma and profiled miRNA expression associated with tumorigenesis. According to their findings, studies performed on paired frozen and FFPE samples showed very similar results. Moreover, a comparison study of microarray, deep sequencing, and RT-PCR methodologies also showed a high correlation between the three technologies. This demonstrated that FFPE samples can be reliably used for miRNA deep sequencing analysis and that future large-scale clinic-based studies may be possible.

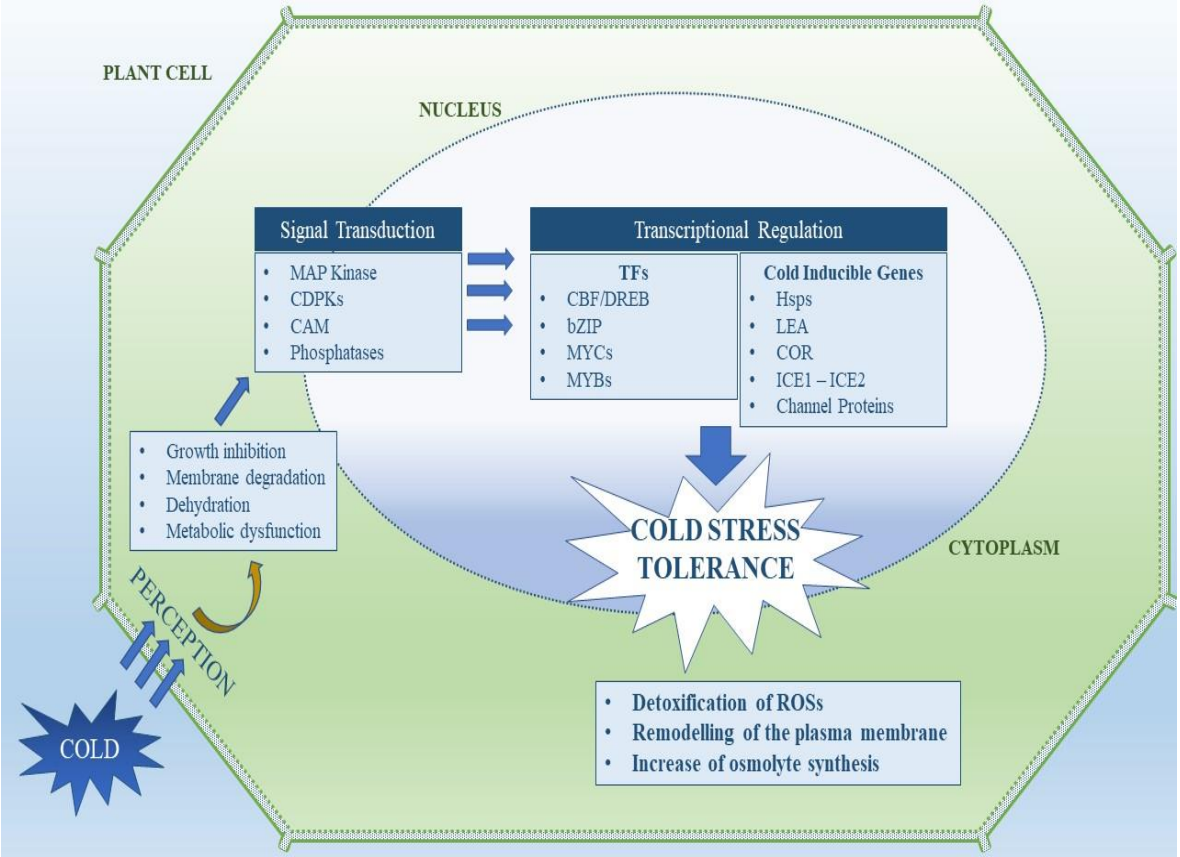

Figure 2. The general molecular response of a plant cell to cold stress.

In the case of plants, differential expression levels of 10 selected miRNAs and their known target genes were assessed in *A. thaliana* with two tested germination times (24 h and 48 h) according to different stages of cryoprepervation (osmoprotection, dehydration, rapid cooling and warming, dilution, and recovery) (26). Six miRNAs (miR393, miR397, miR398, miR159, miR169 and miR172) showed a mixed pattern in 48-h germinated seedlings whereas six miRNAs (miR397, miR398, miR159, miR169, miR172 and miR408) were significantly down-regulated in OD (Osmoprotection-Dehydration) and LD (Liquid nitrogen-Dilution) samples in comparison to the control in 72-h germinated seedlings. Moreover, miR393 was up-regulated 15-fold in LN treated 48-h seedlings while relatively lower induction (only two-fold) was recorded in cryopreserved 72-h seedlings. This shows that, together with elevated antioxidant status and oxidative stress response, the alteration of expression levels of cold-induced genes related-miRNAs (especially miR393) and their target genes play a crucial role in recovery after cryopreservation.

Long non-coding RNAs (LncRNAs)

LncRNAs lack protein-coding function, but are involved in the regulation of gene expression at the epigenetic, transcriptional and post-transcriptional levels (77). In general, lncRNAs regulate gene expression by altering chromatin structure, silencing or activating a gene or a family of genes, and in some cases by cis- or trans-regulation of the entire chromosome (direct regulation of a neighboring gene - cis or indirect regulation through a gene product - trans) (78). In addition, they bind to chromatin-modifying proteins that serve as transcriptional co-activators (79, 80) or form RNA dimers with mRNA sequences to block transcription-associated regions (81, 82). Therefore, the assessment of lncRNA expression profiles could also give clues to understand the biological processes involved in cryopreservation.

The transcriptomes of eight-cell embryos from 11 women undergoing in vitro fertilization treatment were analyzed by single-cell RNA-Seq (83). The results showed that cryopreservation after vitrification did not affect mRNA and lncRNA expression profiles in human embryos,

but that the vitrification-thawing procedure led to minor changes in the transcriptome. Moreover, the roles of potential target genes of differentially expressed lncRNAs in sperm freezability were also investigated by Fraser et al. (84) with the comparison of the differentially expressed lncRNAs of spermatozoa from wild boars classified as having good and poor semen freezability. Differentially expressed lncRNAs were found to be upregulated in pigs in the group with poor semen freezability and significantly affected the response of sperm to cryopreservation conditions. These lncRNAs in both groups were found to have potential cis- and trans-regulatory effects on different protein-coding genes such as *COX7A2L*, *TXNDC8*, and *SOX-7*. Gene Ontology enrichment revealed that these lncRNA target genes associated with numerous biological processes, including signal transduction, stress response, apoptosis, motility and embryo development.

CONCLUSION AND FUTURE PROSPECTS

For the cryopreservation of mammalian and plant cells, multiple stresses to which cells are exposed before and/or after cryopreservation can cause cryoinjuries leading to the loss of viability and/or increased genetic instability. Up to now, with the utilization of transcriptomic analyses in cryopreservation studies, it has been found that numerous genes involved in stress metabolism are activated, which resulted in the accumulation of soluble sugars, proline, betaine, polyamines, phenylpropanoids, and antioxidants in both intracellular and intercellular spaces. Moreover, the accumulation of these stress response biochemicals in cells, which are defined by omic technologies, could also overcome the harmful effects during and after cryopreservation. Hence, cryogenic applications using an optimized protocol that allows the obtainment of living cells with highly preserved cell surface and properly altered gene expression levels could provide the highest cell viability and proliferation rates after cryopreservation. In this context, analysis based on transcriptomic technologies have the potential to enable the development of more effective and efficient protocols in the cryopreservation of all types of cells and tissues in the near future. Moreover, it can also aid the non-invasive monitoring of immune actions and to diagnose the stage of the inflammatory process in mammalian kidney diseases such as lupus nephritis.

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