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Review

Polyamine-dependent gene expression

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Abstract. The polyamines spermidine and spermine along with the diamine putrescine are involved in many cellular processes, including chromatin condensation, maintenance of DNA structure, RNA processing, translation and protein activation. The polyamines influence the formation of compacted chromatin and have a well-established role in DNA aggregation. Polyamines are used in the posttranslational modification of eukaryotic initiation factor 5A, which regulates the transport and processing of specific RNA. The polyamines also participate in a novel RNA-decoding mechanism, a translational frameshift, of at least two known genes, the TY1 transposon and mammalian antizyme. Polyamines are crucial for their own regulation and are involved in feedback mechanisms affecting both polyamine synthesis and catabolism. Recently, it has become apparent that the polyamines are able to influence the action of the protein kinase casein kinase 2. Here we address several roles of polyamines in gene expression.

Key words. Polyamines; RNA; gene expression; casein kinase 2; aggregation.

Physiological role of polyamines

The polyamines are ubiquitous low-molecular weight aliphatic cations. Spermine was first observed as microscopic crystalline inclusions in human seminal fluid during the 17th century by Lewenhoeck [1]. Several genes involved in polyamine synthesis are essential for cell viability [2] and mammalian development [3]. Polyamines have been implicated in a large number of cellular processes, including functioning of ion channels [4], nucleic acid packaging, DNA replication, apoptosis, transcription and translation [5]. These multivalent cations can also act as electrostatic bridges between the phosphate charges of DNA and RNA as well as a variety of other highly charged linear chains (e.g. actin filaments and microtubules) [6].

Cells have evolved mechanisms to ensure the tight regulation of intracellular polyamine pools. Insufficient levels of polyamines result in subopitmal growth and, in some cases, cell death, including apoptosis. Unregulated metabolism resulting in high levels of polyamines can lead to cellular transformation. Extremely high polyamine contents are toxic to cells and can trigger apoptosis [7].
The diamine putrescine and the polyamines spermidine and spermine are products of amino acid decarboxylation. Some bacteria derive putrescine from arginine via a two-step decarboxylation and urea-liberating mechanism [8]. Other prokaryotes, and all eukaryotes, derive putrescine from ornithine via the action of ornithine decarboxylase (ODC). Ornithine is either formed from arginine by arginase-catalyzed hydrolysis or imported from exogenous sources. Putrescine is subsequently converted to spermidine by the addition of a propyl amine group, which is donated by decarboxylated *S*-adenosylmethionine. This series of reactions is catalyzed by *S*-adenosyl-

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methionine decarboxylase (SAMDC) and spermidine synthase. The conversion of spermidine to spermine also requires SAMDC along with spermine synthase (see fig. 1).

Like their amino acid precursors, polyamines can also be imported [9]. Bacteria have a number of genes dedicated to the uptake of these amines from the environment [10]. Although a polyamine transporter has been identified on the membrane of vacuoles in *Saccharomyces cerevisiae,* genes encoding polyamine transporters on the plasma membrane in eukaryotes have not yet been identified. The main exogenous source of polyamines in mammals is the intestinal lumen. Putrescine is present in the human diet in high amounts [11, 12], primarily in foods of plant origin. Spermidine and spermine, however, are more often found in meat and meat products [12].

The catabolism of polyamines begins with the acetylation of spermidine and spermine by spermidine/spermine N^1-acetyltransferase (SSAT), producing N^1-acetylspermidine, N^1-acetyl spermine and N^1, N^{12}-diacetylspermine [13]. Certain of these acetylpolyamines are substrates for export from the cell via the diamine exporter [14]. Oxidation of acetylpolyamines by the flavin adenine dinucleotide (FAD)-dependent polyamine oxidase is a mechanism to reconstitute shorter-chain amines along with 3-acetamidopropanol and hydrogen peroxide, from longer-chain polyamines [15]. In mammals, polyamine synthesis and uptake are regulated by a negative feedback mechanism initiated by a novel polyamine-dependent ribosomal frameshifting mechanism to be addressed below.

Many of the enzymes involved in polyamine metabolism have also been shown to be essential for growth and development. In *S. cerevisiae* that lack the gene for *S*-adenosylmethionine decarboxylase, putrescine is made, but not spermidine. These mutants do not grow, and a similar phenotype is seen in mutant strains of *Escherichia coli* and *S. cerevisiae* that are unable to synthesize putrescine [2, 16]. In mice with a disrupted SAMDC gene, homozygous embryos did not develop past embryonic day 6.5 [17], indicating the requirement for this enzyme in cellular proliferation. In *Leishmania donovani,* SAMDC null mutants could not grow without exogenous polyamines; spermidine was able to rescue this phenotype [18]. Mice generated with a disrupted ornithine decarboxylase (ODC) gene failed to develop after embryonic day 3.5 [3]. Chinese hamster ovary cells that are de-

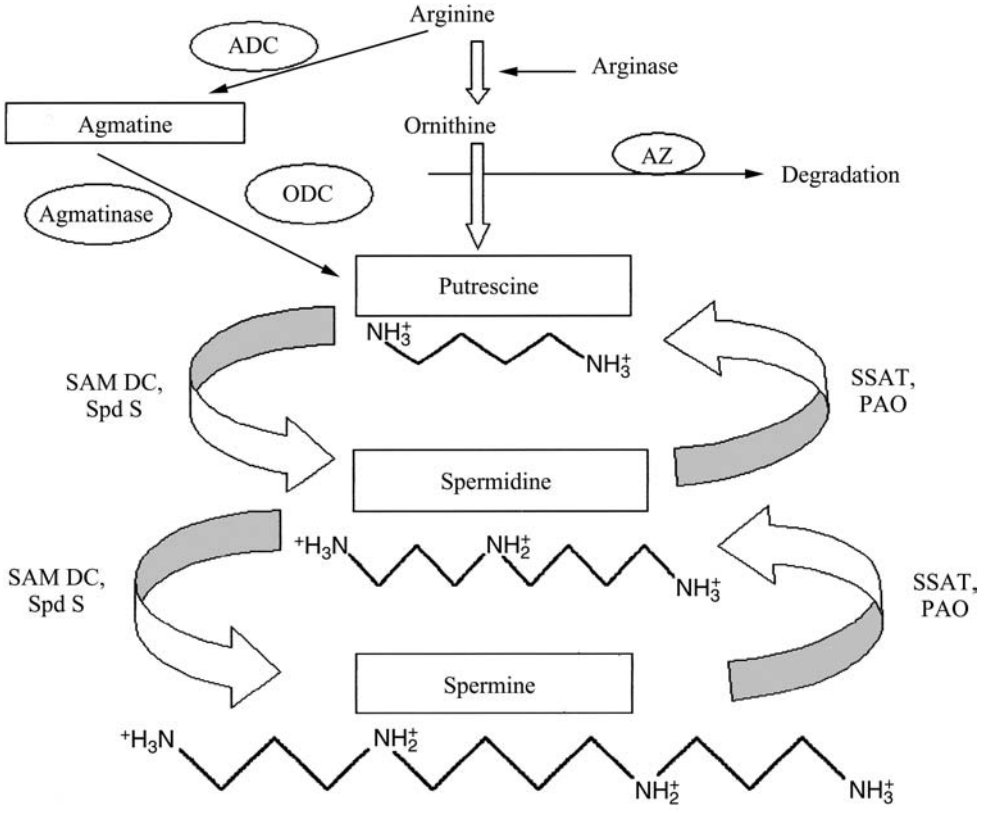

Figure 1. Polyamine metabolism from arginine to spermine. Arigine is converted to agmatine by agmatine decarboxylase. Agmatine is then converted to putrescine by agmatinase. Alternatively, ornithine decarboxylase (ODC) converts ornithine to putrescine. ODC can be degraded by antizyme. *S*-Adenosylmethionine decarboxylase (SAMDC), spermidine (spd) and spermine (spm) synthase convert putrescine to spermidine and spermidine to spermine, respectively. Spermidine/spermine N^1-acetyltransferase (SSAT) and polyamine oxidase (PAO) work together in the catabolism of spermine and spermidine.

ficient in ODC are depleted of putrescine in 24 h and of spermidine after 48 h. The cells begin to die within 5–6 days of stopping polyamine supplementation [19].

The polyamines influence cell phenotypes via several mechanisms [20]. One unique mechanism involves the polyamine-dependent posttranslational formation of the amino acid hypusine in the putative eukaryotic translation initiation factor eIF5A [21] (fig. 2). This review will focus on the polyamines and their involvement in gene expression, including the effects on RNA and DNA processing as well as posttranslational effects and functional activation of proteins.

Effect of polyamines on DNA conformation and chromatin condensation

In most biological systems DNA occurs in a highly condensed form. This condensation can occur because of space restriction and/or by condensation agents such as polyamines. These multivalent cations act as electrostatic bridges between DNA and phosphate charges [22]. DNA has been shown to precipitate in the presence of natural and synthetic polyamines. DNA aggregates can form in very dilute monomolecular DNA solutions with long DNA strands; however, in the case of multimolecular DNA, aggregate formation occurs only in highly concentrated solution irrespective of strand length [23]. The condensates induced by polyamines show toroidal or rodlike shapes when observed under the electron microscope [24]. Spermidine precipitation happens when there is one spermidine molecule per eight nucleotides. At this concentration of spermidine a complete neutralization of histone-positive charges and chromatin phosphate charges occurs [25].

At higher concentration of polyamines these aggregates resolubilize. Maximum aggregation is seen with triplex DNA followed by duplex. Single-stranded DNA (ss DNA) shows the least aggregation amongst the three. ssDNA is the easiest to resolubilize, followed by duplex DNA and triplex DNA, requires the highest concentration of polyamines for resolubilization. Presence of sodium in the medium has a negative effect on DNA precipitation. Even in the resoubilized state spermine stabilizes the DNA. When synthetic polyamines were used for the same study, it was evident that pentamines were more effective than tetramines and triamines. At low sodium concentration spermidine was the most effectively resolubilized triamine, compared with its derivatives [26]. Saminathan et al. also found that the precipitation phenomenon depends on ionic concentration of the medium, but resolubilization is hardly affected by ionic strength. Spermidine or spermine in the concentration range of 1 µg/ml–1 mg/ml can result in aggregation of calf thymus DNA fragments of around 146 bp [27]. These aggregates are anisotropic and have cholesterine liquid-crystalline phases. The con-

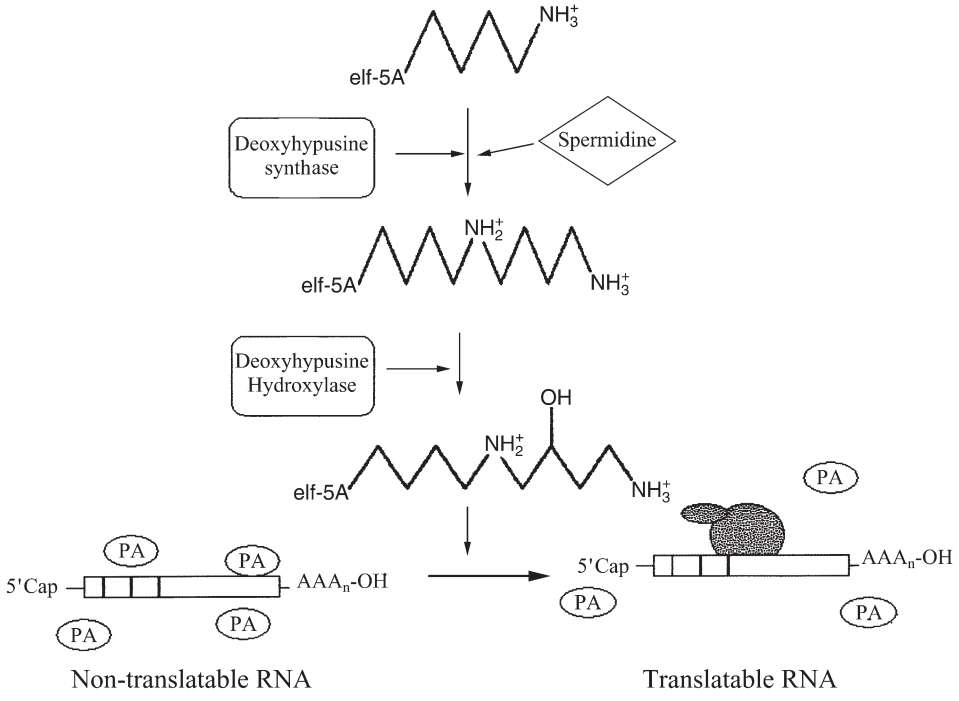

Figure 2. eIF5A contains a lysine residue that is converted to deoxyhypusine in a spermidine-dependent reaction by deoxyhypusine synthase. Deoxyhypusine is then converted to hypusine by deoxyhypusine hydroxylase eIF5A acts to counteract the polyamine (PA) aggregation of specific RNAs. Without eIF5A, specific RNA cannot be translated or degraded.

densation and resolubilization of DNA is of great biological significance. Many theories have been proposed to explain the aggregation and resolubilization. Raspaud et al. [22] used an ion-bridging model to explain this phenomenon. At high DNA concentrations, all multivalent cations become condensed on the DNA strand, and monovalent ions exist in both condensed and free state. In this regime, as mentioned earlier, DNA strand length does not matter. For intermediate DNA concentrations, aggregate formation does not depend on either of the qualities, i.e. it is independent of polymer length and added monovalent cation concentration. This state would exist only if all multivalent cations were condensed and none of the monovalent cations were condensed. For low DNA concentrations, DNA strand length and monovalent cation concentration matter, as precipitation occurs with only multivalent cations condensed on the DNA strand and some multivalent cations and all monovalent cations free in the solution [22].

The polyamines have also been shown to induce a conformational change of DNA from the B form to the Z form [28]. Hasan et al. [28] showed that in the presence of polyamines, calf thymus DNA exists in the Z conformation. The authors used anti-Z-DNA antibodies to show that without the presence of polyamines, there is very little DNA found in the Z conformation. Interestingly, polyamine analogue induction of Z conformation has been shown to inversely correlate with the cytotoxicity of *cis*-diaminedichloroplatinum (II) in human brain tumor cell lines [29].

Polyamines have been shown to have a preference for DNA structure. All polyamines show preference in stabilizing A-DNA compared with B-DNA. The major groove of A-DNA is the site preferred by all polyamines. In B-DNA putrescine prefers the sugar-phosphate backbone as the binding site, whereas spermidine and spermine bind along the backbone and in the major or minor groove [30].

Preferential binding sites for each polyamine have been proposed based on spectral analysis. According to Raman spectra, putrescine, which by virtue of its size can bind to both the major and minor grooves of DNA, shows preference for the double-stranded DNA (dsDNA) major groove. It has been proposed that putrescine binds a phosphate group and thymine-O2 or a guanine/adenine-N3. These contacts can allow for hydrophobic interaction between methylene groups of dsDNA and putrescine. An exo-groove interaction has been proposed where an amino group from putrescine interacts with two consecutive dsDNA phosphate groups [31]. Putrescine molecules can modify the hydrogen bond pattern of DNA at high concentration and thus can give rise to hypochromism. Putrescine is restricted to intrastrand binding because of its size, but spermidine molecules can have interstrand interactions. Raman data propose that spermidine would preferentially bind the DNA minor groove. For the second amino group of spermidine there are two interstrand interaction possibilities. Spermidine can interact with pyrimidine-O2 or purine N-3. The inner amino group of the molecule can interact with the thymine-O2 site. There is the possibility of hydrophobic interactions, too. The interstrand distance between phosphate moieties at the DNA major groove does not allow for interstrand interaction with spermidine at this site. Spermine has been the most difficult molecule in terms of studying these interactions and still has been one of high interest amongst researchers. The Raman spectra also show that spermine can interact with the major groove of DNA preferentially. But there is certain amount of evidence showing sequence dependence for these interactions. There seems to be a difference between the interaction intensity between A-T and G-C base pairs. DNA folding over the polyamine molecules has been thought to facilitate the interaction by causing the minor groove to broaden and the major groove to constrict. Exo-groove interaction could be possible for spermine [31].

Polyamines have been found to induce B to Z transition in synthetic polymers. When a synthetic polymer poly(dA-dC)·poly(dG-dT) was allowed to form a complex with polyamines, Z confirmation was induced in the polymer. This was evident when the polymer-polyamine complex produced anti-Z antibodies in experimental animals [32]. It has been shown that spermidine and spermine can induce a left-handed Z-DNA formation in short stretches of $(dG\text{-}dC)_n$ sequences found in right-handed B-DNA. These short stretches are found in many organisms and thus might have significance in terms of gene regulation [33].

Polyamines have been shown to increase the stability of core nucleosomes [34] and to aid in vitro in condensation of the 10-nm fiber to the 30 nm [35]. An in vivo model has also shown that polyamines affect chromatin structure. When Snyder et al. treated HeLa cells with DL-α-difluoromethylornithine (DFMO), methylglyoxal bis (guanylhydrazone) or a combination of the two, the accessibility of chromatin by DNAase I and II was increased [36]. Histone acetyltransferase that acetylates histone H4 can acetylate spermidine. This could be of biological significance in terms of inducing gene expression [37]. Polyamines might be involved in chromatin remodeling, as they have been shown to influence histone acetylation. When K6/ODC and ODC/Ras transgenic mouse models were used for studying the effect of polyamines on histone acetylation, it was seen that activities of histone acetylase and histone deacetylase were increased in ODC-overexpressing skin cells and isolated keratinocytes. When these cells were treated with DFMO, this effect was reversed, indicating that polyamines are involved in either direct or indirect regulation of chromatin remodeling. It was seen that in these mouse mod-

els where ODC was overexpressed, histone acetylase enzyme showed preference for Lys-12 in the tail domain of histone H4 [38].

Polyamines are also known to have an effect on topoisomerase II function. When Chinese hamster ovary cells were treated with two different polyamine inhibitors, it was seen that topo II was unable to cleave DNA. The enzyme was not directly affected, as it retained its activity in crude nuclear extracts. Topo II activity depends on DNA structure, and polyamine depletion can cause DNA structure to change. So polyamines might not be interacting with topo II directly but might be influencing its function by altering DNA structure. Thus polyamines are of great significance to maintain the structural balance of DNA and chromatin [39].

Polyamines and DNA stability

As mentioned earlier, polyamines participate in electrostatic interaction with DNA and thus can stabilize the B-form family of DNA because of the difference in association constants. If there are alternating pyrine-pyrimidine sequences on each strand, the left-handed, Z form of DNA is stabilized by polyamines [37]. Spermidine and spermine have been shown to favor the formation of triplex DNA and also to stabilize them at neutral pH [40]. Antony et al. synthesized cyclopolyamines and studied their effect on DNA stabilization. These cyclopolyamines were synthesized by combining linear polyamines with a $-(CH_2)_n$-bridge ($n = 3, 4, 5$). They observed a differential stabilization effect. [4,3]-spermine and [4,4]-spermine had a stabilizing effect on the triplex DNA formed by a purine-motif triplex-forming oligonucleotide. But [4,4]-putrescine and [4,5]-putrescine as well as [4,5]-spermine had no stabilization effect on these oligonucleotides [41]. All the polyamines have been shown to stabilize triplex DNA formation preferentially. Putrescine homologues have also been observed to have a stabilizing effect on triplex DNA [42]. Antony et al. have shown that spermine and spermine homologues stabilize triplex DNA while not having any effect on the underlying duplex structure. There was an increase in the T_m for triplex DNA when these molecules were added, but there was no effect on the T_m for duplex DNA [43].

The effect of polyamines and their synthetic homologues as well as their acetyl derivatives were studied on triple- and double-stranded structures. These structures were made from poly(dA) and poly(dT). Melting temperatures for these structures were measured in the presence and absence of polyamines. When polyamines were present, two transitions were seen. During the first transition period triplex DNA melted to duplex or ssDNA, and in the second transition period the duplex DNA melted away. In the presence of 0.5 mM putrescine the T_m values for both transition periods were higher compared with the T_m values found in its absence. Also, triplex DNA could not be detected when putrescine was absent. The T_{m2} value, which is the melting temperature during the second transition, was even higher when samples were treated with 2.5 μM spermidine or 0.1 μM spermine. When polyamine concentration was increased, both melting transitions became one and gave the T_m value corresponding to the melting of triplex DNA to ssDNA. These results can be of great significance in the field of gene regulation research, especially when triplex DNA is used to inhibit or suppress transcription of specific genes [42].

It has been recently found that polyamines exist in aggregates in the nucleus of replicating Caco-2 cells. Polyamines are found to interact with phosphate anions by forming ionic bonds. These are called nuclear aggregates of polyamines (NAPs). These NAPs carry a net positive charge and thus bind to phosphate groups on the DNA strand. There are three different NAPs known so far. They have molecular weights of 8000, 4800 and <1000. These NAPs are found in the nucleus of many different cell types. NAPs are rich in spermidine and spermine. These aggregates might induce conformational changes in the DNA structure and also might have protective or stabilizing effects on DNA [44]. It is already known that the 4800-Da NAP is absent in differentiated Caco-2 cells that do not replicate after confluency. This suggests that 4800-Da NAP has a significant role in replication. The 8000-Da NAP is found in both differentiated and replicating cells. This aggregate could be playing a role in DNA stabilization and protection [45].

Polyamines can stabilize synthetically generated DNA duplexes, which have abnormal sequences. In presence of polyamines the T_m values of these duplexes were elevated. Spermine is found to introduce sequence-specific changes in conformations of oligonucleotides. Polyamines were also found to stabilize DNA with mismatched and bulged base pairs. In the presence of polyamines, their k_d values decreased and their T_m values increased [46].

It has been observed that spermine and putrescine bind preferentially to matrix-associated sites of DNA-protein cross-link formation. By doing this they either stop DNA protein interaction or scavenge hydroxyl radicals interacting with these sites. Spermine is more effective in this function than putrescine [47].

Polyamines are also known to protect DNA from the strand breaks caused by reactive oxygen species generation. Spermine protects DNA at the physiological concentration of 1 mM. Spermidine, putrescine and some of their acetylated derivatives were tested for similar protective effects but were found to have no effect on intrastrand breakage caused by reactive oxygen species [48].

Polyamines have a protective effect against degradation of DNA base pairs upon exposure to γ rays. This could be

because of compaction of DNA by polyamines, thus reducing the accessibility of DNA to hydroxyl ions or any other DNA-damaging agent. Binding of polyamines to DNA is the most important feature in terms of protective effects. It has also been observed that the protection occurs for all the regions of the DNA to the same extent whether it be major or minor groove [49].

Spermine and spermidine have been studied for their effect on stabilization of different DNA duplex structures. Different DNA duplexes were designed. These included perfect, mismatched, bulged-loop and hairpin-like structures. It was seen that in the presence of polyamines or salts the T_m value for all these structures increased. Surface plasmon resonance study was carried out to find out whether there was any cation-specific effect on duplex formation. It was found that duplex formation occurred with same efficiency irrespective of the cation used. Spermine was most effective in stabilization of these structures, followed by spermidine. All the other cations used were less effective than spermidine. The duplex structures were stabilized both kinetically and thermodynamically in the presence of polyamines. This could be of significance in tumor cells, where elevated levels of polyamines could protect mismatched base pairs from DNA repair enzymes, thereby allowing mutations in many important genes [46].

Site-specific recombinases such as Cre recombinase are important for carrying out genetic manipulations such as conditional gene ablation in mice. The *Cre-lox* system is becoming popular amongst researchers because of its specificity and precision in recognizing the target sites. A recent study using the surface plasmon resonance technique indicates that *Cre-loxP* interaction can be affected by spermidine concentration. The surface plasmon resonance system is an automated technique which can perform real-time analysis of biomolecular interactions. This technique has been widely used to detect protein-protein, DNA-protein, protein-lipid and protein-substrate interactions. In the presence of spermidine the wt-Cre affinity for *loxP* half-site decreases, but at the same time the affinity for the entire *loxP* site increases. This change in affinity leads to an increase in the cooperative moment of the *Cre-loxP* interaction. This increase could be the result of change in association rates, as the off rates were constant over the spermidine range used. This phenomenon can be explained on the basis of spermidine-DNA interaction, which can result in DNA bending in the major groove. DNA bending will lead to a closed conformation of all the major grooves and thus will lead to bending of the *loxP* site. Cre binds with the major groove of DNA while interacting with *loxP*. In the presence of spermine, the association velocity between Cre and its binding site, which is an inverted repeat element of *loxP,* will decrease because of the narrowing of *Cre-lox* interfaces. Spermidine's effect on DNA can bring two inverted repeats of *loxP* closer and induce protein-protein interaction. This study indicates that the presence of agents such as spermidine, which can cause structural changes in DNA, can have a great effect on the association of Cre with *loxP* without actually affecting its dissociation pattern [50].

Polyamines and casein kinase 2

Despite what we know about the high level of conservation of the serine/threonine protein kinase casein kinase 2 (CK2), and the lethal effect of gene disruption [51], the function of CK2 in cells remains a mystery. This enzyme, however, is undoubtedly influenced by the presence of polyamines. CK2 binding to DNA is stimulated by the presence of polyamines [52], and the presence of polyamines can stimulate phosphorylation of a variety of substrates by CK2 [53]. This protein contains two α-catalytic subunits and two regulatory β subunits. The protein contains a highly acidic region that has been proposed to be involved in the interaction with polyamines [54].

Although it has been shown that CK2 binds DNA through its α subunit [52], spermine analogues were used to establish that polyamines interact directly with the CK2 β subunit, and that replacing glutamine 60, 61 and 63 of the β subunit with alanine residues leads to loss of stimulation of CK2 activity by spermine. The alanine substitutions also lead to decreased spermine binding affinity for casein kinase [55]. It was seen that spermine was most efficient in enhancing kinase activity and also served as the most efficient ligand for CK2. A fusion protein was made of maltose-binding protein (MBP) and the β subunit region containing Asp51–Pro110. This protein was used to study its binding with spermine as the function of ionic strength. It was observed that Asp51–Pro110 is the region containing an autonomous domain which is functional in binding with the four positive charges of spermine [56].

When ODC was overexpressed in intact Balb/MK cells, CK2 activity as well as CK2 protein levels increased as expected. Along with this redistribution of CK2, protein was also observed in these cells. By performing immunofluorescence microscopy, it was shown that CK2 migrated from the cytoplasm to the nucleus. Also, when ODC is overexpressed in transgenic mice, CK2 activity increases. One can then hypothesize that an increase in ODC activity might lead to an increase in CK2 activity, which can then act in favor of transformation of cells to malignancy [57].

On binding with CK2, spermine can bring about a change in the quaternary structure of the enzyme; the structure thus formed corresponds to the catalytically active form of CK2. The sedimentation pattern of CK2 was studied in the presence of 1 mM spermine. It was observed that CK2 sedimented as a mixture of many interconverting oligo-

mers in the absence of spermine, but in the presence of spermine CK2 showed sedimentation as a sharp peak, indicating a ringlike structure. This means that spermine can stabilize CK2 in its ringlike structure by dissociating the high molecular weight oligomers. This transformation to ringlike structure can occur in the absence of peptide substrate and ATP [58].

It is widely believed that CK2 can be stimulated by polycation-substrate interactions. A surface plasmon resonance study has been carried out to find out whether there is any interaction between polylysine and the peptide substrate RRREEETEEE. The study indicates that there is interaction between polylysine and this substrate. It is already known that polylysine binds with CK2. As a result of this binding, the enzyme configuration undergoes a change. Surface plasmon resonance study indicates that polylysine binding with CK2 is biphasic in nature. This biphasic nature of polylysine binding is responsible for formation of high molecular mass complexes which can stimulate CK2 activity. As already mentioned in this section, polyamines can activate CK2 activity. But the mechanism by which polyamines activate is different from polycations such as polylysine. Evidence strongly indicates that spermine binds with CK2 and stabilizes its most active conformation of the enzyme. Also, no peptide-spermine interaction was seen [59].

Lately, a link between one of the substrates of CK2, the Myc oncoprotein, and polyamines has become more established. It is known that myc transactivates ornithine decarboxylase. As polyamine concentration increases intracellularly, CK2 phosphorylation of Myc also increases [53].

Polyamines and proteins

Polyamines not only bind to DNA and RNA but can also sometimes bind with proteins. They can affect protein function either by interacting with them directly or indirectly by influencing their binding with DNA and so on. Polyamines have been observed to influence neuroactive proteins such as transmitter receptors. Polyamines can form electrostatic interactions with anionic sites on many different macromolecules, including proteins. Spermine and spermidine can modulate the function of *N*-methyl-D-aspartate (NMDA) receptors in a positive or negative fashion. They can bind with extracellular sites and thereby activate the NMDA receptors or with ion channel sites and inhibit them. Generally, extracellular concentrations are enough to activate these receptors but not high enough to inhibit the NMDA receptors. Under certain circumstances, such as brain injury, polyamine levels might increase and cause inhibition of these receptors. Polyamines are also known to interact with many other transmitter receptors, but it has been difficult to find their sites of interaction on these proteins because of their low affinity for ionotropic receptors. These interactions are effected by the length and charge of the polyamine involved [60].

Polyamines can have a significant effect on proteins required for transcription initiation and so on, where DNA bending is required. Polyamines are known to bend DNA and thus might regulate expression of many genes. The effect of polyamines has been studied on estrogen receptors (ERs) and their response elements (EREs). At a concentration of 100–500 μM, spermine increases ER-ERE interaction by two- to threefold. But at a higher concentration of around 1000 μM, a decrease in ER-ERE complex formation is seen. This suggests that varying polyamine concentration regulates protein function. A similar increase was observed when binding of the transcription factor NF-κB with its response element NRE was studied in the presence of polyamines [61].

The effect of polyamines on DNA binding of various transcription factors has been studied. A similar kind of study was carried out for viral protein ICP-4, which is a transcriptional regulator of herpes simplex virus (HSV)-1. It was seen that the binding activity of ICP-4 and many others, such as USF and TEF3, is increased in the presence of spermidine and spermine. This effect was studied on about five to six different proteins, and all of them showed an increase in binding, though at different magnitude. Divalent cations such as Mg^{2+} and Ca^{2+} were tested for a similar effect, but they had no effect on binding of these proteins. It seems that the increase occurs because of increased binding of proteins to their binding sites in the presence of polyamines and not because of any stabilizing effect on DNA-protein complex formation. In millimolar concentrations, spermidine inhibits binding of Oct-1 to DNA. Also, polyamines have different effects on same proteins. For example, spermidine stimulates activity of Ig/enhancer binding protein (EBP), whereas the same is inhibited by millimolar concentrations of spermine [62]. DNA transition from B to Z occurs at a very low concentration of polyamines (micromolar), but in order to have any significant effect on DNA-protein interaction, a much higher concentration of polyamines is required (millimolar) [63].

Polyamines can affect protein degradation as well. They can either increase or decrease the rate at which a particular protein is degraded. Several reports indicate that polyamines inhibit proteases in different plant species. In vitro studies have also indicated that polyamines increase resistance to some proteinases by changing their conformational state [64].

Polyamines have been shown to affect long-lived and short-lived proteins in human embryo lung cells (L-132). In these cells, rates of proteolysis were greatly affected by increasing or decreasing the level of polyamines. In polyamine-depleted cells, proteolysis of long-lived pro-

teins decreased, and their half-life increased considerably. Exactly the opposite effect was observed in short-lived proteins, as both their degradation rate increased and their half-life decreased. Whether polyamines have a direct effect on proteolytic enzymes and pathways or modulate their function indirectly still remains to be determined [65].

eIF5A and RNA processing

Eukaryotic initiation factor 5A is a small, acidic protein which contains 154 amino acids and has a molecular mass of 16.7 kDa [66]. eIF5A is required for cell proliferation; an increase in cells arrested in G1 is seen when yeast is depleted of eIF5A [67]. eIF5A is the single known protein that contains the novel amino acid hypusine, and modification of the eIF5A into its hypusine-containing form is also essential for cell viability [68].

eIF5A is dependent upon spermidine for modification into a fully functional protein (i.e. synthesis of the hypusine residue). In the first step of this process, deoxyhypusine synthase catalyzes the transfer of the butylamine moiety of spermidine to an amino group of a specific lysine residue of the eIF5A precursor to form deoxyhypusine and free 1,3-diaminopropane [69, 70]. Deoxyhypusine hydroxylase then β-hydroxylates the side chain of deoxyhypusine and completes the hypusine modification and eIF5A maturation. Despite many years of research, eIF5A's function has been controversial, although a correlation between formation of the modified eIF5A and cell proliferation has been observed [71–74].

This protein was originally thought to be a translation initiation factor based on its in vitro ability to stimulate methionyl puromycin synthesis, which mimics the formation of the first peptide bond in protein synthesis [75]. However, when yeast is depleted of eIF5A, only a 30% reduction in the rate of protein synthesis is seen [67], arguing against eIF5A being an initiation factor for general protein synthesis.

The amino acid sequences of both eIF5A and deoxyhypusine synthase are highly conserved among many eukaryotic species and are functionally conserved throughout eukaryotic evolution [76–78]. It has been shown that deoxyhypusine synthase is also essential for cell viability in yeast [79]. The amino acid sequence of this enzyme is highly conserved and exists as a homotetramer from 40–43 kDa. Inhibition of either deoxyhypusine synthase [80] or deoxyhypusine hydroxylase [81] leads to antiproliferative effects.

Many studies have attempted to elucidate the function of eIF5A. Rev is the first example of an RNA that is processed via an eIF5A-dependent mechanism. Rev is a viral protein that is required for HIV-1 gene expression [82]. Rev shuttles between the nucleus and the cytoplasm of host cells to mediate the translocation of unspliced and incompletely spliced viral messenger RNAs (mRNAs) across the nuclear envelope. Rev contains an effector domain that contains four critically spaced leucine residues. eIF5A was shown to bind to this domain of Rev [83], and blocking the expression of eIF5A using antisense techniques blocked Rev function, again implicating eIF5A in RNA processing.

A temperature-sensitive mutant of the yeast form of eIF5A, TIF51A, which encodes a serine-to-proline change, exhibited a defect in mRNA decay and accumulated uncapped mRNAs, as well as showed a 30% decrease in protein synthesis at the restrictive temperature [84]. The yeast accumulated both nonsense-containing and wild-type mRNAs, and the authors proposed that the mutation could affect mRNA decay either at or downstream of decapping [84]. Recently, a temperature-sensitive allele of the yeast TIF51A was characterized and shown to contain a mutation in each of the two domains of the protein. This mutation caused a defect in the decay of mRNA degradation, but overexpression of six genes allowed growth of this mutant. The suppressor genes included PAB1, PKC1 as well as regulators of PKC1 [85]. Interestingly, PAB1 is involved in many aspects of RNA metabolism, including decay, translation and processing.

Several examples of polyamine-dependent RNAs exist in mammals. Matrilysin is a member of the matrix metalloproteinase family and has been implicated in invasion in several epithelial tissues. Treatment of colon tumor cells with the specific pharmacological inhibitor of ODC, DFMO, reduces extracellular levels of matrilysin protein after 4 days, an effect that was shown to be independent of steady-state mRNA levels and of mRNA stability [86]. Recently, cycloxygenase-2 (COX-2) RNA, which contains eIF5A response elements, was shown to be influenced by polyamine depletion. When DFMO is used to deplete the polyamines, there is a subsequent induction of COX-2 protein, with, however, only a minimal effect on COX-2 RNA levels [87]. The COX-2 3′-untranslated region (UTR) contains sequences recognized by eIF5A, as reported by Xu and Chen [88]. Studies showed that the DFMO-dependent change in COX-2 protein was mimicked by the eIF5A response elements contained in the COX-2 RNA 3′ UTR [87].

It has long been known that polyamines are necessary for stabilization and for crystallization of transfer RNAs (tRNAs) [89, 90]. It was recently shown that spermine binding to 16s ribosomal RNA (rRNA) can induce conformational changes [91], confirming an independent effect of the polyamines on RNA structure.

Translation of some RNAs may be affected by the ability of polyamines to bind to RNAs. Polyamines can cause aggregation of specific RNAs, a phenomenon that is perhaps dependent on the structure of the RNA. eIF5A, which has been shown to bind specific sequences in the

RNA [88], appears to inhibit the aggregation of the RNAs [A. Childs, unpublished data] (see fig. 2). Apparently, eIF5A can reverse the effects that polyamines have on translation of particular RNAs.

Translational frameshifting

Polyamines are known to affect translational frameshifting in at least two specific cases. Although exact mechanisms have yet to be elucidated, we know that these critical shifts of the ribosome are important in polyamine metabolism and in expression of the transposable element Ty. The Ty1 transposable element is found in yeast and uses a +1 ribosomal frameshift to produce the TYA-TYB fusion protein [92]. Polyamine depletion has been shown to increase the +1 frameshift efficiency, leading to increased transposition of the Ty1 transposon [93] and a subsequent increase in expression of the gag-pol protein. In null SPE2 yeast mutants that cannot make spermidine, there is an increase in +1 frameshifting efficiency that responds to the Ty1 frameshift sequence [94].

Antizyme, so called because it is the 'anti-enzyme for ODC' [95] is a 26.5-kDa protein that targets ODC for decarboxylation by the 26S proteosome. ODC is induced when cells are stimulated to grow by various mitogens [96–99]. Antizyme is able to prevent accumulation of excessive polyamines. ODC can also contribute to the induction of apoptosis in response to growth factor withdrawal [100, 101]. However, coexpression of ODC and antizyme in human fibroblasts rescued this phenotype [102]. Polyamine levels are kept in check by stimulating translation of antizyme mRNA, and transcription of the antizyme gene decreases when cells are depleted of polyamines [103]. Antizyme mRNA contains two overlapping open reading frames (ORFs). The first contains two methionines, but a frameshift is necessary to move the ribosome into the second ORF and for the translation of a functional protein [104]. The frameshift must occur one base before the stop codon of the first ORF. The following six codons are also critical to stimulate frameshifting, as is a pseudoknot that follows the stop codon [105] (see fig. 3). The polyamines are thought to increase the efficiency of the ribosomal frameshift, thereby increasing their negative regulator levels in the cells [106]. Formation of the pseudoknot, however, is not dependent on the polyamines [107]. The specific frameshift mechanism is system dependent (i.e. +1 in reticulocyte lysates and –2 in *Saccharomyces cerevisiae*). Antizyme not only targets ODC for degradation but can also suppress the uptake of polyamines by cells [108, 109].

There is an inhibitor of antizyme that binds it with higher affinity than ODC, known as antizyme inhibitor [110]. Antizyme inhibitor is encoded by an ODC-related gene but does not have enzymatic activity [110]. Antizyme inhibitor is thought to stabilize newly synthesized ODC, preventing its degradation by the 26S proteosome. Antizyme inhibitor is induced in growth-stimulated mouse fibroblasts, and transient transfections with an antizyme inhibitor-expressing construct leads to reactivation of ODC [111].

Translation

S-Adenosylmethionine decarboxylase is a key enzyme in the regulation of spermidine and spermine. It has recently been established that a variety of growth factors can affect SAMDC expression and that in H-ras transformed cell lines SAMDC plays an important role in malignant progression [112].

Synthesis of SAMDC is regulated at the level of translation by spermidine and spermine. The mRNA of SAMDC contains an upstream open reading frame that encodes a short peptide with the sequence MAGDIS. This peptide

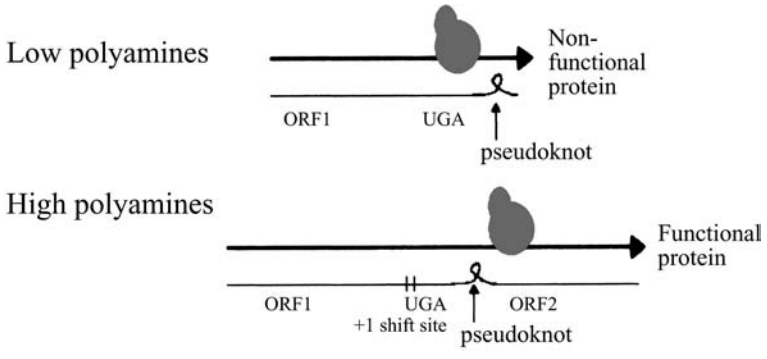

Figure 3. Antizyme translation. When low levels of polyamines are present in the cell, the ribosome stalls at the weak stop codon, UGA, and translation is terminated. When levels of polyamines are higher, the ribosome stalls at the weak codon, but the polyamines help to facilitate movement of the ribosome past the stop codon and the pseudoknot.

suppresses translation of the downstream cistron in a polyamine-dependent mechanism. High levels of polyamines, but not $MgCl_2$, lead to less production of this peptide and a subsequent increase in SAMDC [113]. Ruan et al. [114] have shown that the ORF of the peptide is 14 nucleotides from the cap and can be moved further from the cap without affecting translation of the peptide or polyamine control of the translation. It has also been shown that the peptide can induce polyamine-dependent translation when inserted at the 5′ end of human growth hormone mRNA.

It has also been shown that this peptide's actions are very sequence specific. Using a yeast suppressor screen, Mize et al. have shown that only aspartic acid at codon 4 will ensure suppression of the downstream cistron. In order to maintain function, only valine could be substituted for the isoleucine found at codon 5 [115]. Changing the fourth or fifth amino acid led to a significant decrease in the peptide's sensitivity to polyamine concentration [113]. The most recent evidence indicates that elevated levels of polyamines may block peptide synthesis by stabilizing a stalled ribosome near the termination codon. Raney et al. [116] showed that the peptidyl-tRNA molecule is associated with the ribosome and the nascent peptide, suggesting that polyamine regulation occurs after peptidyl-tRNA formation but before hydrolysis of the peptidyl-tRNA bond.

Summary

Polyamines influence gene expression via several distinct mechanisms. Polyamines affect DNA conformation as well as chromatin condensation. Interestingly, at higher polyamine concentrations, polyamines are able to resolubilize chromatin, exposing DNA and possibly allowing access to the transcription and replication machinery. Polyamines can also activate the protein kinase CK2, which is known to phosphorylate a plethora of substrates, including *c*-myc. The polyamine-modified protein eIF5A is essential for viability in eukaryotes and apparently acts to influence RNA's availability for translation. Polyamines are able to induce a ribosomal +1 frameshift on the antizyme RNA and in the Ty1 RNA, allowing translation of functional proteins. *S*-adenosylmethionine decarboxylase translation is also influenced by the polyamines through an interaction between the ribosome and an upstream peptide. Polyamines are able to influence gene expression not only through direct interaction with nucleic acids but also through protein modifications and through apparent sequence-specific interaction with DNA.

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