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Kinetin — 45 years on

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Abstract

Kinetin (N^6-furfuryladenine) was the first cytokinin to be isolated almost 45 years ago from DNA as an artifactual rearrangement product of the autoclaving process. Since then its chemical structure and properties have been well described. Most importantly, a wide variety of biological effects of kinetin, including those on gene expression, on inhibition of auxin action, on stimulation of calcium flux, on cell cycle, and as an anti-stress and anti-ageing compound have been reported. Recently, views on this very well known plant growth factor have undergone substantial modifications. New data have appeared which show that kinetin is formed in cellular DNA as the product of the oxidative, secondary modification of DNA. Although the biological significance of the endogenous kinetin and the molecular mechanisms of its action are not completely understood at present, most of the experimental data point toward kinetin acting as a strong antioxidant in vitro and in vivo, with potential beneficial uses in agriculture and human healthcare.

Keywords: Cytokinin; Reactive oxygen species; Oxidative damage; Anti-ageing

1. Introduction

Kinetin (N^6-furfuryladenine) was the first cytokinin isolated and identified in 1955 [1,2]. Cytokinin is the generic name used to designate a plant-growth substance that promotes cell division and may play a role in cell differentiation. Most commonly, cytokinins comprise a group of N^6-substituted adenine derivatives that induce division and organogenesis in plant cell cultures and affect other physiological and developmental processes. However, despite almost a half century of study, virtually nothing has been revealed about the mechanisms that mediate a variety of responses to cytokinins in general and kinetin in particular. There is also a view that the effects of cytokinins are never exclusively the result of cytokinin action, but are rather co-mediated by other factors and hormones, since synergistic, antagonistic and additive interactions between them have been observed. Although several general reviews on cytokinins have been published [3–6], we consider that there are at least three main reasons which justify a review focusing entirely on kinetin as timely. These are: (i) the recent identification of kinetin in cellular DNA and plant tissue extracts; (ii) the new data on the biological properties of kinetin; and (iii) the commercial applications of kinetin in cosmeceuticals. Here we review data on the structure, properties and mechanism of action of kinetin along with a discussion of the major issues yet to be resolved.

2. Structure, chemical properties and natural occurrence

N^6-furfuryladenine or kinetin was isolated for the first time in 1955 from autoclaved herring sperm DNA and has been thought to be an artificial DNA rearrangement product [1,2]. As the

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purine derivative (Fig. 1A) it can be easily split off DNA at slightly acidic conditions due to the ability of its glycosylic bond. Kinetin is an amphoteric compound with pK_a values 4 and 10. It is dissolved in strong acids, alkalis, and glacial acetic acid, is slightly soluble in ethanol, butanol, acetone and ether, but is practically insoluble in distilled water. It sublimes at 220°C at atmospheric pressure and is unaffected by autoclaving either at pH 0.5 or 12 [2].

Crystals of kinetin suitable for X-ray analysis have been obtained by slow cooling of a hot ethanol solution. The structure of kinetin was refined to a R index of 0.06. The bond angles and distances of adenine moiety are in good agreement with the corresponding values of the adenine with exception of the C(6)–(6) bond which is slightly longer in kinetin. The molecular conformation of kinetin can be best characterised in term of two planes; one passing through the adenine moiety and the other through the furfuryl group. The dihedral angle between the two planes is 79°. Since the N(6)-substituent is distal to the imidazole ring, hydrogen bonding of the Watson–Crick type is not seen in crystals of the base. In contrast, the orientation of the substituent leads to the Hoogs-

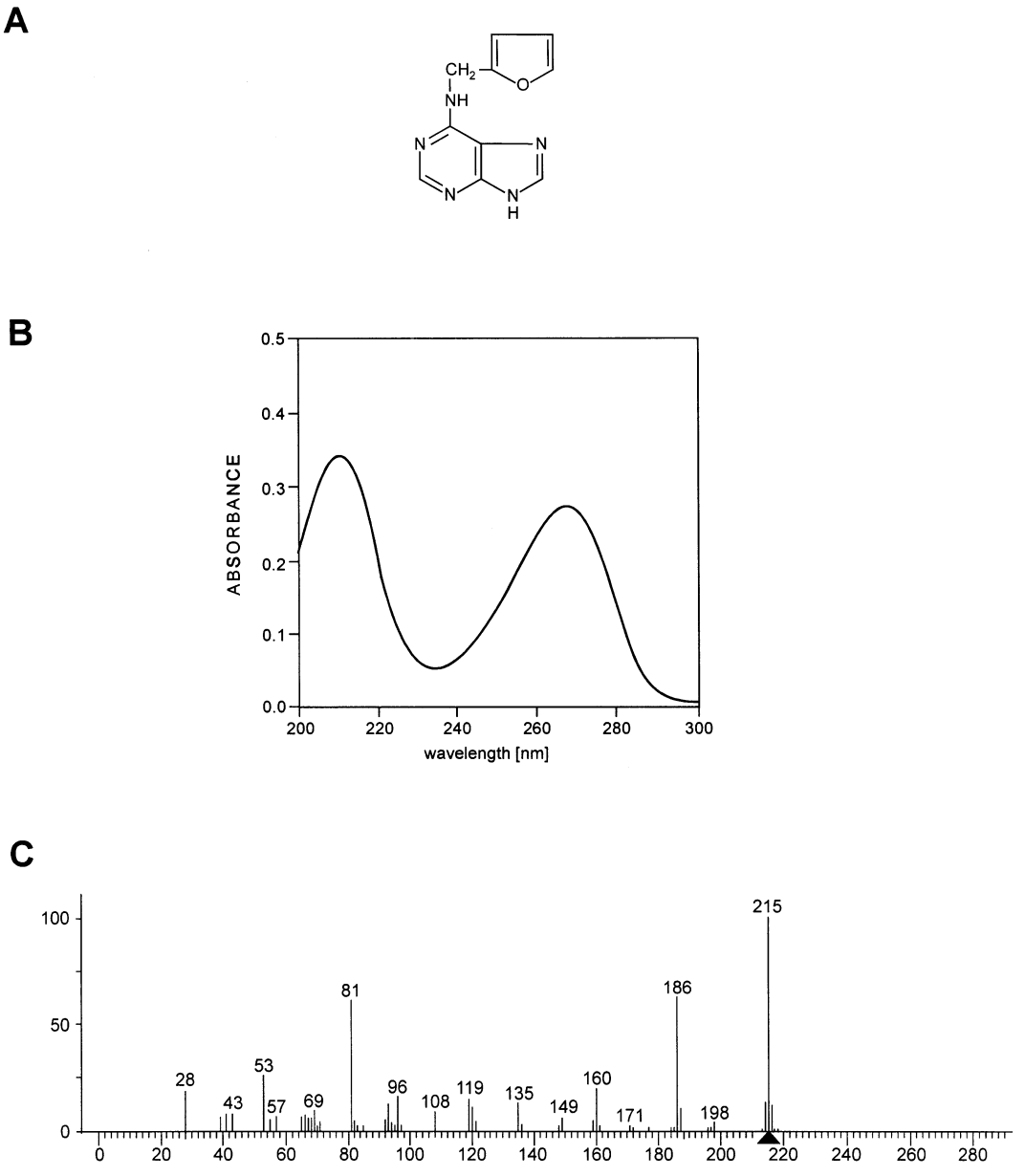

Fig. 1. N6-furfuryladenine or kinetin. (A) Chemical formula; (B) UV spectrum; and (C) mass spectrum.

teen type of base pairing across crystallographic centres of inversion by N6–H…N7 and N9–H…N3 hydrogen bonds resulting in continuous ribbons of purine bases with the furfuryl ring pointing up and down from these sheets [7,8]. The distal orientation of the N6-substituent with respect to the imidazole ring is similar to other substituted N6 adenines. An interesting consequence of Hoogsten type bonding is a hydrogen bond interaction between C(8)–H(8)…0(15) at 2.64 Å. Any substituent on N1 significantly restricts conformational freedom of the N6–CH2 bond because of the steric repulsion of these positions [7–9].

A significant contribution to identification of kinetin in natural products was provided by discovering its electrochemical properties [10]. This gave the first approach for a chromatographic analysis of kinetin in DNA and cell extracts using an HPLC system with an electrochemical detector. It is known that the basic components of nucleic acids are not electrochemically active at potentials of about 300–700 mV. The electrochemical properties of kinetin at about 650–900 mV potential are due to the presence of the furfuryl substituent at the exocyclic amine group of purine [10]. Similarly, the guanine residue after modification at C8 with the hydroxyl group (OH) acquires electrochemical properties, which are currently used for identification of 8-oxodeoxyguanosine (8oxodG) in DNA at the level of four residues of 8-oxo-dG per 10^6 deoxyguanosine residues [11].

The crucial evidence for the presence of N^6-furfuryladenine in natural products came from the mass spectrometric analysis of DNA components (Fig. 1C). The molecular signal of 215 m/e has been identified and the pattern of mass signals interpreted [10]. The spectra obtained for an isolated product and that of N^6-furfuryladenine already deposited in the mass spectra library at USA National Institute of Standards and Technology were identical [12,13]. The presence of kinetin in DNA has been identified also by GC/MS analysis of trimethylsilyl (TMS) derivatives of nucleic acids bases, obtained after acidic hydrolysis of DNA. Kinetin modified with one or two TMS groups showed the m/e signals of 287 and 359, respectively [Dizdaroglu, personal communication]. Recently, new examples of kinetin's presence in natural products have been reported [14,15]. The mass spectrometry and HPLC analyses showed kinetin in an extract of the root nodules of *Casuarina equisetifolia*, produced by the inoculation of *Frankia*. Actinomyces that make up the genus Frankia are distinguished by their ability to induce N-fixing root nodules on certain non-leguminous plants [14]. Also palmarosa (*Cymbopogon martinii* var. *motia*) roots contain 6-furfuryladenine, the amount of which increased significantly after inoculation of *Glomus* species [15].

The finding of kinetin in DNA and cell extracts raised the obvious question about its synthetic pathway. Furfural has been suggested as a putative precursor of kinetin [13]. It has been found that furfural is formed during hydroxyl radical oxidation of the C5′ of deoxyribose in DNA [16,17]. This aldehyde has also been found among many of the reaction products of metallophorphyrins with DNA [18]. The C5′ radical is formed by H5′ abstraction from the deoxyribose residue of DNA in addition to an oxidative attack at C1′, which constitutes the principal mechanism of that reaction [17]. The calculated amount of furfural (C5′ hydroxylation reaction) relative to 5-methylene-2-furanone (C1′ hydroxylation derivative) catalysed by phorphirins was found to be 15% and was suggested that furfural residue is one of the primary products of hydroxy radical damage of DNA.

Those mechanistic considerations have been based on the bleomycin mechanism or on product analysis of DNA constituents that were treated with ionising radiation. Recently, deuterium kinetic isotope effects on the rate of cleavage of DNA by the hydroxyl radical were measured. These experiments demonstrate that the hydroxyl radical reacts with the various hydrogen atoms of the deoxyribose in the order $5'H > 4'H > 3'H = 2'H = 1'H$ [19,20]. This order of reactivity not only parallels the exposure to solvent of the deoxyribose hydrogens, but also provides information on the mechanism of DNA damage. It is clear that the C5′ hydroxylation and spontaneous cleavage of the DNA backbone with β-elimination leads to the furfural formation. The presence of furfural in the extracts of various cells was confirmed by its reaction with 0-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride [21,22]. The oxime derivative obtained was then converted to TMS derivatives and analysed by mass spectrometry, which confirmed the furfural presence in the cell extracts [23]. The reaction of furfural with plasmid

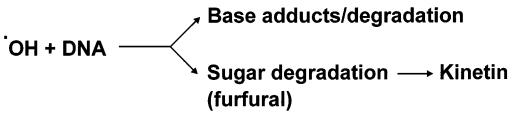

Fig. 2. Consequences of hydroxyl radical interaction with the DNA can be either base modification and adduct formation, or oxidation of deoxyribose leading to the formation of furfural, and kinetin as a secondary oxidation product.

DNA and AT-rich oligonucleotides leads to destabilisation of DNA secondary structure. Although the reaction products have not been analysed this suggests the formation of a bulky modification in DNA [24,25]. Once furfural is formed in the vicinity of DNA, it can efficiently react with the exocyclic amino groups of DNA components and can form the Schiff base with adenine residues (Fig. 2), and possibly with cytosine moieties. Furthermore, dehydration and reduction of the intermediate leads to formation of kinetin at the level of DNA [23].

3. Biological properties

Data regarding the biological properties of kinetin are scattered throughout literature, often in combination with studies on the effects of other cytokinins. However, here we focus on kinetin with respect to its effects on gene expression, on inhibition of auxin action, on stimulation of calcium flux, on cell cycle, and as an anti-stress and anti-ageing molecule. Although many of these effects are also reported for other cytokinins, our aim here is to review the data in relation to kinetin only.

3.1. Effects on transcription

The ability of kinetin to stimulate transcription initiation has been demonstrated in *Arabidopsis thaliana* directly at the rRNA gene promoter [26]. Kinetin treatment induced a significant increase in nascent RNA polymerase-I transcripts in dose- and time-dependent manners. Whereas rRNA transcript levels were increased several fold per unit of total RNA in response to higher exogenous concentrations, the transcripts from the ribulose biphosphate carboxylase gene family were unaffected or even reduced at high concentrations of kinetin [26]. This means that the increased steady-state level of rRNA transcripts is not part of a general positive response to cytokinin. Because rRNA transcription is similarly induced in roots, floral tissues and whole plants it suggests that kinetin action is not tissue specific [26]. As gibberellic acid, abscisic acid, auxin and ethylene had no detectable effect on rRNA transcription [26], kinetin may be therefore the main molecular regulator of transcription and hence the growth status in plant cells. In line with this observation is the fact that kinetin activates the major nucleolar organiser region in the basal, equatorial and near-apical tissue through increasing its size and changes in morphologies from round or oval to elongated-oval and dumbbell shaped [27].

It has also been shown that kinetin enhances incorporation of 8-[^{14}C]-adenine into DNA, RNA, poly A^+ of embryos and cotyledons and thus increases the germinating capacity of the seeds [28]. However, a close relationship between the DNA and RNA biosynthesis of embryos and cotyledons and the ability of the seeds to germinate and their embryos to continue growing is not obvious. On the other hand kinetin is incorporated into tobacco callus RNA preparations. Approximately 0.7% of the radioactive kinetin was recovered in ribosomal RNA and tRNA preparations, of which the rRNA fraction contained 90% of the incorporated kinetin [29]. However, it has also been reported that kinetin inhibits incorporation of [^{3}H]-uracil and [^{14}C]-leucine by tobacco cells suspension culture [30]. Furthermore, it has been shown that radioactive kinetin was incorporated site-specifically into prokaryotic and eukaryotic tRNAs [31]. This reaction is probably catalysed by a tRNA-transglycosylase in similar synthetic pathway as queosine in tRNA. Incorporation of kinetin into tRNA takes place at position 37 (a position next to the 3′ end of the anticodon triplet), which is normally occupied with modified adenine bases having cytokinin activity [31].

3.2. Cell cycle control

As in other eukaryotes, the cell cycle in plants depends on kinase activities which are modulated by cyclins. Catalytic activity of the cyclin-dependent kinase (cdc2) in explants increases after treatment with kinetin. In suspension of *Nicotiana plumbaginifolia*, kinetin was stringently required only in late G2 phase of the cell division cycle (cdc) and cells lacking kinetin were arrested in G2

phase with inactive p34cdc2-like histone kinase [32]. Control of the cdc2 kinase by inhibitory tyrosine phosphorylation has been indicated by a high phospho-tyrosine amount in the inactive enzyme of arrested pith. Kinetin stimulated the removal of phosphate, activation of the enzyme and rapid synchronous entry into mitosis [32]. It means that plants can control cell division by tyrosine phosphorylation of cdc2 by coupling this mitotic control to hormonal signal.

Kinetin promotes an increased formation of haploid ascospores in *Saccharomyces cerevisiae*. In yeast, many genes and their products involved in cell-cycle control have been characterised. These include mostly adenyl cyclase and cAMP dependent and independent kinases. Yeast sporulation is a developmentally regulated process that depends on extracellular cues and a cascade of intracellular responses, including protein phosphorylation and leads to production of haploid ascospores. In experimental tests sporulation can be induced in nitrogen-poor medium containing a non-fermentable carbon source. Although the mechanism of kinetin action is not known in this context, it somehow influences this reaction chain [33]. Furthermore, kinetin induces stomatol opening in *Tradescantia albiflora* and its pathway operates via guanylate cyclase upregulation. The stomatal opening is reversibly inhibited by inhibitors of cGMP cyclase [34]. Kinetin also showed positive isotropic effects in rat atria by P2-purinoceptors as well as modification of cGMP [35]. Studies on the intestinal absorption of a kinetin β-glucoside in rat showed that both glucosides are stable and are not transported in the upper region of the small intestine by Na^+/nucleoside cotransporter [36].

3.3. Effects on calcium flux

Kinetin induces vegetative bud formation in the moss *Physcomitrella patens* which is an integral part of the moss life cycle leading to the development of the mature gametophore essential for subsequent sexual reproduction. The plant hormone applied to moss cells causes profuse premature bud formation and a localised increase in Ca(II) from 250 to 750 nM [37]. It takes place after addition of kinetin but precedes the cytokinin-induced cell division [37]. These studies also indicate that cytokinin-modulated calcium entry takes place via dihydropyridine (DHP)-sensitive channels in the plasma membrane [38]. Low levels of kinetin stimulate binding of a calcium channel blocker, azidopine (arylazide 1,4-dihydropyridine) in a manner qualitatively similar to its ability to stimulate calcium influx into moss protoplasts. Because the effect of kinetin on binding has been observed without preincubation of hormone with the membranes, it seems therefore that kinetin stimulates calcium influx through the plasma-membrane Ca(II) channel, stimulates azidopine binding with the channel, alters its conformation and facilitates inhibitor binding [37,38].

3.4. Inhibition of auxin action and anti-stress effects

Kinetin represses abscisic acid (ABA)-induced accumulation, throughout the plant *Spirodela polyrrhiza*, of cDNA TUR2 transcripts encoding a homologue of yeast ATP-binding cassette transporter involved in the ATP-dependent efflux of a variety of structurally unrelated cytotoxic compounds [39,40]. Environmental stress conditions such as low temperature and high salt lead also to elevated levels of the TUR2 transcript which is manifested by formation of dormant buds (turions) in *S. polyrrhiza* [40]. Interestingly, an environmental stress and ABA up-regulate also mitogen activated protein kinase (MAPK) genes [41]. Similarly the elevated levels of mRNA transcripts (TUR4) of *S. polyrrhiza* encoding a novel basic peroxidase localised to the cell wall, induced with ABA are inhibited by kinetin [39]. Kinetin not only totally inhibits the induction of the turions by ABA, but also alleviates ABA-induced growth inhibition [39,40].

A potential complexity in the mode of action of two growth regulators, kinetin and ABA, which interact antagonistically in many cases, could suggest that kinetin functions as an anti-stress agent [39]. Such a conclusion can be drawn from the conversion reaction of linolenic acid to jasmonic acid in a lipooxygenase (LOX)-dependent octadecanoid pathway. Kinetin treatment lowered the lipooxygenase activity, whereas ABA increased it. The cytokinin acts in this case by preventing formation of reactive oxygen species (ROS) or as a direct radical scavenger [42].

One of many defensive systems in plants against pathogen attack is the production of secondary metabolites, e.g. phytoalexins mediated by jas-

monic acid (JA). Kinetin counteracts the phytoalexin production probably as an effective free radical scavenger and inhibits a hypersensitive response [43]. This is supported by the observation that ascorbic acid, as a potent active oxygen species generator, enhances the JA-inducible phytoalexin production and that kinetin protects the cell somehow against stress [43]. Exogenously applied kinetin is able to suppress viral necrosis and necrosis of plant cells caused by mercuric chloride [44]. It could mean that the increased cytokinin level is involved in the induced resistance as measured by reduction of development of necrotic lesions. Thus it appears that the first stress in the lower leaves may induce resistance to the second stress in the upper leaves by increasing their cytokinin level, which in some way suppresses the necrotic reaction of these issues. In this context kinetin could be considered to induce systemic acquired resistance and be regarded as an anti-stress hormone in the plant [44–46]. Some cytokinins are known to modify plant respiration and this has been considered to be due to the inhibition of electron flow from internally generated NADH to oxygen via a rotenone-sensitive dehydrogenase and the cytochrome pathway. When deamino NADH, a selective substrate for rotenone-sensitive dehydrogenase, was used as an electron donor, the inhibition rate by kinetin was about 45% of the respiration at 400 μM ($I_{50} = 580$ μM) similar to that effected by isopentenyladenine [47]. In tobacco cell culture (*Nicotiana tabaccum L.*) phenylalanine: ammonia lyase (PAL) activity was induced in response to exogenously added kinetin [48].

3.5. Anti-ageing effects

Kinetin is well known for its anti-ageing effects in plants [3–6,49]. Recently, however, its strong anti-ageing effects on human skin cells and fruitflies have been also reported [50–52]. For example, it was shown that kinetin delays the onset of several cellular and biochemical characteristics associated with cellular ageing in human skin fibroblast cultures [50]. These ageing characteristics include alterations in morphology, increase in cellular protein content, and accumulation of protein–lipid–peroxide conjugates in the cytoplasm as indicated by the extent of cytoplasmic autofluorescence [50]. Other effects of kinetin on the inhibition of growth of human fibroblasts, epithelium and mammary carcinoma have also been reported [53–55]. In other studies it was shown that kinetin slows down development and ageing, and prolongs the lifespan of the fruitfly *Zaprionus paravittiger* mainly due to a reduction in age-specific death rates throughout the adult lifespan [51]. Furthermore, an increase in the specific activity of catalase during developmental stages and in adult insects has been observed [52]. Based on the results obtained from studies on the anti-ageing effects of kinetin on human skin cells [56], and from dermatological tests against photo-ageing (Senetek plc, personal communication) several skin care products and potential cosmeceutical formulations containing kinetin are being developed.

4. Mechanisms of action

From an analysis of the wide ranging effects of kinetin it is clear that kinetin functions at transcriptional, translational, post-translational and metabolic levels. Although the mechanisms of action of kinetin at all these levels is yet to be revealed, various lines of evidence, including the stimulation of transcription, cytoplasmic calcium(II) influx and MAP kinase pathways indicate that kinetin is involved in signal transduction and may also act as an anti-oxidant. As a signalling molecule, kinetin may stimulate other defence pathways, such as DNA repair and proteosome-mediated protein turnover. Recently, kinetin has been shown to activate *Arabidopsis* cell division through induction of the D-type cyclin CycD3 [57]. Indirect evidence that kinetin reduces the extent of the age-related accumulation of proteins in human fibroblasts indicates its mode of action by stimulating protein turnover pathways [50].

In an analysis of the antioxidative character of kinetin as a free radical scavenger one could consider two possibilities: oxygen radicals can directly abstract hydrogen from the α-carbon of the amine bond of N^6-furfuryladenine; or they can undergo faster dismutation reaction in aqueous solution when kinetin is complexed with copper. The preferred N(3)/N(9) copper(II)binding mode of kinetin in Cu(II)-kinetin complex has been observed [58]. H-bridging and π-stacking in the tetrameric structure occurs. The complex has a weak

ferromagnetic exchange [58]. The rate constants of reaction of kinetin-Cu(II) complex with O^{2-} determined by polarography at pH 9.8 is 2.3×10^7 m^{-1} [59,60].

A direct effect of kinetin on superoxide dismutase activity (SOD) has been observed in plants. There are two SODs: Fe- and CuZn- containing enzymes of chloroplasts of *N. tobaccum* encoded by the nuclear genes sodB and sodCp, respectively. They exhibited a different expression pattern upon oxidative stress and treatment with different hormones including kinetin. The sodCp mRNA level decreased by 50% and sodB mRNA level was threefold higher in response to kinetin [61]. These differences in the expression pattern indicate that both enzymes have different functions in an autooxidative system. Recent studies have shown that kinetin protects DNA from hydrogen peroxide-induced formation of 8-oxodG by the Fenton reaction in vitro [62]. The involved mechanism is unknown but kinetin could prevent the hydroxyl radical-mediated DNA damage either by acting as a radical scavenger or by binding iron in such a way that it is no longer a Fenton reductant or in way that prevents iron from associating with the DNA. Further studies are required to resolve this issue.

Another possibility of kinetin's mode of action is through its effects on the cell membrane [42] and on the intracellular calcium flux [37,38]. At the membrane level, kinetin inhibits the release of linolenic acid and its conversion to jasmonic acid in a lipooxygenase-dependent octadecanoid pathway [42]. In this case too, kinetin appears to act by preventing the formation of reactive oxygen species or as a direct radical scavenger [42].

5. Issues remaining be resolved

Although kinetin was the first cytokinin to be identified, comparatively much less is known about this multipotent molecule as compared with other cytokinins. In the review above we have given an overview of the literature regarding the structure, function, and chemical and biological properties of kinetin. Admittedly, our understanding of kinetin's biological significance, effects and mechanisms is too meagre to reach any definitive conclusions. There are several crucial issues yet to be addressed and resolved some of which are:

- What is the biological significance of the natural formation of kinetin in the DNA?
- What are the levels of kinetin formation in the DNA from various organisms and ages?
- Is kinetin, as a base modification within the DNA, mutagenic?
- Is kinetin repaired or removed from the DNA?
- Can kinetin nucleotide be (re)incorporated during DNA synthesis?
- Can exogenously supplied kinetin become incorporated in replicating DNA?
- What is the nature of kinetin receptors, if any?; and
- What are the intracellular interactions of kinetin with other macromolecules including RNA and proteins?

Forty-five years on and so little is known about this molecule! With its wide ranging biological effects in yeast, plants and animals including human cells, and the possibilities of it being developed as a potential cosmeceutical, kinetin deserves and demands extensive research in order to elucidate its mechanisms of action and interaction in biological systems.

References

[1] C.O. Miller, F. Skoog, M.H. von Saltza, F.M. Strong, Kinetin, a cell division factor from deoxyribonucleic acid, J. Amer. Chem. Soc. 77 (1955) 1392.
[2] C.O. Miller, F. Skoog, F.S. Okumura, M.H. von Saltza, F.M. Strong, Isolation, structure and synthesis of kinetin, a substrate promoting cell division, J. Amer. Chem. Soc. 78 (1956) 1375–1380.
[3] O.S. Letham, L.M.S. Palni, The biosynthesis and metabolism of cytokinins, Ann. Rev. Plant Physiol. Plant Mol. Biol. 34 (1983) 163–197.
[4] M. Kaminek, Progress in cytokinin research, Trends Biotech. 10 (1992) 159–164.
[5] A.N. Binns, Cytokinin accumulation and action: biochemical, genetic and molecular approaches, Ann. Rev. Plant Physiol. Plant Mol. Biol. 45 (1994) 173–196.
[6] Mok DWS, Mok MC, editors. Cytokinins: Chemistry, Activity and Function. Boca Raton, FL: CRC Press, 1994.
[7] M. Soriano-Garcia, R. Parthasarathy, Structure-activity relationship of cytokinins: Crystal structure and conformation of 6-furfurylaminopurine (kinetin), Biochem. Biophys. Res. Commun. 64 (1975) 1062–1068.
[8] M. Soriano-Garcia, R. Parthasarathy, Stereochemistry and hydrogen bonding of cytokinins: 6-furfurylaminopurine (kinetin), Acta Cryst. B33 (1977) 2674–2677.
[9] D.I.C. Scopes, U. Zarnack, N.J. Leonard, R.Y. Schmitz, F. Skoog, Alternative routes for the genesis of kinetin: a synthetic intramolecular route from 2′-deoxyadenosine to kinetin, Phytochemistry 15 (1976) 1523–1526.

[10] J. Barciszewski, G.E. Siboska, B.O. Pedersen, B.F.C. Clark, S.I.S. Rattan, Evidence for the presence of kinetin in DNA and cell extracts, FEBS Lett. 393 (1996) 197–200.
[11] H. Helbock, K.B. Beckman, M.K. Shigenaga, P.B. Walter, A.A. Woodall, H.C. Yeo, et al., DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine, Proc. Natl. Acad. Sci. USA 95 (1998) 288–293.
[12] Standard Reference Data Program, US Dept. of Commerce, Technology Administration, National Institut of Standards and Technology. Gaithensburg, M.D. 20899. Edition January, 1995.
[13] J. Barciszewski, G.E. Siboska, B.O. Pedersen, B.F.C. Clark, S.I.S. Rattan, A mechanism for the in vitro formation of N6-furfuryladenine, kinetin as a secondary oxidative damage product of DNA, FEBS Lett. 414 (1997) 457–460.
[14] N. Raman, S. Elumalai, Presence of cytokinin in the root nodules of *Casuarina equisetifolia*, Ind. J. Exp. Biol. 34 (1996) 577–580.
[15] N. Ratti, K.K. Jonardhanan, Effect on growth, phosphorus and cytokinin contents of palmarosa (*Cymbopogon martini* var. *motia*) by *Glomus* inoculation, Ind. J. Exp. Biol. 34 (1996) 1126–1128.
[16] G. Pratviel, M. Pitie, J. Bernadou, B. Meunier, Furfural as a marker of DNA cleavage by hydroxylation at the 5′ carbon of deoxyribose, Angew. Chem. Int. Ed. Engl. 30 (1991) 702–704.
[17] G. Pratviel, M. Pitie, J. Bernadou, B. Meunier, Mechanism of DNA cleavage by cationic manganese porphyrins: hydroxylations at the 1′-carbon and 5′-carbon atoms of deoxyriboses as initial damages, Nucl. Acids Res. 19 (1991) 6283–6288.
[18] G. Pratviel, M. Pitie, J. Bernadou, B. Meunier, Carbon-hydrogen bonds of DNA sugar units as targets for chemical nucleases and drugs, Angew. Chem. Int. Ed. Engl. 34 (1995) 746–769.
[19] B. Balasubramanian, W.K. Pogozelski, T.D. Tulluis, DNA strand by the hydroxyl radical is governed by the accessible surface areas of the hydrogen atoms of the DNA backbone, Proc. Natl. Acad. Sci. USA 95 (1998) 9738–9743.
[20] W.K. Pogozelski, T.D. Tulluis, Oxidative strand scission of nucleic acids: routes initiated by hydrogen abstraction from the sugar moiety, Chem. Rev. 98 (1998) 1089–1107.
[21] T. Nambara, K. Kigasawa, T. Iwata, M. Ibuki, A new type of derivative for electron capture-GC/MS chromatography of ketosteroids, J. Chromatogr. 114 (1975) 81–86.
[22] B.A. Bruenner, A.D. Jones, J.B. German, Simultaneous determination of multiple aldehydes in biological tissues and fluids using gas chromatography/stable isotope dilution mass spectrometry, Anal. Biochem. 241 (1996) 212–219.
[23] J. Barciszewski, G.E. Siboska, B.O. Pedersen, B.F.C. Clark, S.I.S. Rattan, Furfural, a precursor of the cytokinin hormone kinetin, and base propenals are formed by hydroxyl radical damage of DNA, Biochem. Biophys. Res. Commun. 238 (1997) 317–319.
[24] Q.A. Khan, S.M. Hadi, Effect of furfural on plasmid DNA, Biochem. Mol. Biol. Int. 29 (1993) 1153–1160.
[25] S. Uddin, S.M. Hadi, Reactions of furfural and methylfurfural with DNA, Biochem. Mol. Biol. Int. 35 (1995) 185–195.
[26] R.J. Gaudino, C.S. Pikkard, Cytokinin induction of RNA polymerase I transcription in *Arabidopsis thaliana*, J. Biol. Chem. 272 (1997) 6799–6804.
[27] C.S. Karagiannis, A.J. Pappelis, Effect of abscisic acid, gibberellic acid, indoleacetic acid and kinetin on selective ribosomal cistron regulation in quesient and senescent onion leaf base tissue, Mech. Age. Dev. 20 (1994) 145–155.
[28] C. Morales, R.M. Cusido, M. Serrano, Kinetin and the germinating capacity of *Lupinus multilupa* seeds, Rev. Esp. Fisiol. 43 (1987) 87–93.
[29] N. Murai, B.J. Taller, D.J. Amstrong, F. Skoog, Kinetin incorporated into tobacco callus ribosomal RNA and transfer RNA preparations, Plant Physiol. 60 (1977) 197–202.
[30] U. Nudel, E.S. Bamberger, Kinetin inhibition of [^{3}H]-uracil and [^{14}C]-leucine incorporation by tobacco cells in suspension culture, Plant Physiol. 47 (1971) 400–403.
[31] J. Barciszewski, D. Otzen, S.I.S. Rattan, B.F.C. Clark, Specific incorporation of kinetin into eukaryotic and procaryotic transfer ribonucleic acids molecules, Biochem. Int. 28 (1992) 805–811.
[32] K. Zhang, D.S. Letham, P.C. John, Cytokinin controls the cell cycle at mitosis by stimulating the tyrosine dephosphorylation and activation of p34cdc-2-like H1 histone kinase, Planta 200 (1996) 2–12.
[33] H.M. Laten, Cytokinins affect spore formation but not cell division in the yeast *Saccharomyces cerevisiae*, Biochim. Biophys. Acta 1266 (1995) 45–49.
[34] M. Pharmavati, T. Billington, C.A. Gehring, Stomal guard cell responses to kinetin and natriuretic peptides are cGMP-dependent, Cell. Mol. Life Sci. 54 (1998) 272–276.
[35] G. Froldi, U. Gallo, E. Ragazzi, L. Caparrotta, 6-benzylaminopurine: a plant derived cytokinin inducing positive ionotropism by P2-purinoceptors, Planta Med. 65 (1999) 245–249.
[36] T. Mizuma, S. Masubuchi, S Awazu, Intestinal absorption of acyclovir-(-glucoside: comparative study with acyclovir, guanosine and kinetin-?-glucoside, Pharm. Res. 16 (1999) 69–73.
[37] K.S. Schumaker, M.J. Gizinski, Cytokinin stimulates dihydropyridine-sensitive calcium uptake in moss protoplasts, Proc. Natl. Acad. Sci. USA 90 (1993) 10937–10941.
[38] K.S. Schumaker, M.J. Gizinski, 1,4-dihydropyridine binding sites in moss plasma membranes, J. Biol. Chem. 270 (1995) 23461–23467.
[39] K. Chaloupkova, C.C. Smart, The abscisic acid induction of a novel peroxidase is antagonised by cytokinin in *Spirodela polyrrhiza* L, Plant Physiol. 105 (1994) 497–507.
[40] C.C. Smart, A.J. Fleming, Hormonal and environmental regulation of a plant PDR5-like ABC transporter, J. Biol. Chem. 271 (1996) 19351–19357.

[41] T. Mizoguchi, K. Ichimura, K. Shinozaki, Environmental stress response in plants: the role of mitogen-activated protein kinases, Trends Biotech. 15 (1997) 15–19.
[42] T.R. Prakash, P.M. Swamy, P. Reddanna, Characterization and behaviour of 15-lipooxygenase during peanut cotyledonary senescence, Biochem. Biophys. Res. Commun. 172 (1990) 462–470.
[43] S. Tamogami, R. Rakwal, O. Kodama, Phytoalexin production elicited by exogenously applied jasmonic acid in rice leaves (*Oryza sativa* L) is under control of cytokinins and ascorbic acid, FEBS Lett. 412 (1997) 61–64.
[44] E. Balazc, B. Barna, Z. Kiraly, Effect of kinetin on lesion development and infection sites in Xanti-nc tobacco infected by TMV: single-cell local lesions, Acta Phytopathol. Acad. Scienti. Hung. 11 (1976) 1–9.
[45] E. Balazc, I. Sziraki, Z. Kiraly, The role of cytokinins in the systemic aquired resistance of tobacco hypersensitive to tobacco mosaic virus, Physiol. Plant Pathol. 11 (1977) 29–37.
[46] I. Sziraki, E. Balazc, Z. Kiraly, Role of different stress in inducing systemin aquired resistence to TMV and increasing cytokinin level in tobacco, Physiol. Plant Pathol. 16 (1980) 277–284.
[47] M. Sue, H. Miyoshi, H. Iwamura, Specific interaction of cytokinins and their analogs with rotenone sensitive intermitochondria, Biosci. Biotech. Biochem. 61 (1997) 1806–1809.
[48] N. Nagai, F. Kitauchi, K. Okamoto, M. Shimosaka, M. Okazaki, A transient increase of phenylalanine ammonia -lyase transcript in kinetin-treated tobacco callus, Biosci. Biotechnol. Biochem. 58 (1994) 558–559.
[49] S. Gan, R.M. Amasino, Cytokinins in plant senescence: from spray and pray to clone and play, BioEssays 18 (1996) 557–565.
[50] S.I.S. Rattan, B.F.C. Clark, Kinetin delays the onset of ageing characteristics in human fibroblasts, Biochem. Biophys. Res. Commun. 201 (1994) 665–672.
[51] S.P. Sharma, P. Kaur, S.I.S. Rattan, Plant growth hormone kinetin delays aging, prolongs the life span and slows down development of the fruifly *Zapronius paravittiger*, Biochem. Biophys. Res. Commun. 216 (1995) 1067–1071.
[52] S.P. Sharma, J. Kaur, S.I.S. Rattan, Increased longevity of kinetin-fed *Zapronius* fruitflies is accompanied by their reduced fecundity and enhanced catalase activity, Biochem. Mol. Biol. Int. 41 (1997) 869–875.
[53] M.F. Orr, B. McSwain, The effect of kinetin upon epithelium and fibroblasts in tissue culture, Cancer 10 (1957) 617–624.
[54] M.F. Orr, B. McSwain, The effect of kinetin, kinetin ribofuranoside and gibberellic acid upon cultures of skin and mammary carcinoma and cystic disease, Cancer Res. 20 (1960) 1362–1364.
[55] E. Kowalska, Influence of kinetin (6-furfurylo-aminopurine) on human fibroblasts in the cell culture, Folia Morphol. 51 (1992) 109–118.
[56] Rattan SIS. Method and composition for ameliorating the adverse effects of aging. United States Patent, No. 5,371,089, 1994.
[57] C. Riou-Khamlichi, R. Huntley, A. Jacqmard, J.A. Murray, Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin, Science 283 (1999) 1541–1544.
[58] M. Parvez, W.J. Birdsall, Structure of a copper(II) kinetin complex, Acta Crystal. C46 (1990) 1775–1778.
[59] H. Inoue, M. Hirobe, Superoxide dismutase mimetic activity of cytokinin-copper(II) complexes, Biochem. Biophys. Res. Commun. 137 (1986) 372–377.
[60] S. Goldstein, G. Czapski, SOD-like activity studies of cytokinin-copper (II) complexes, Free Rad. Res. Commun. 12-13 (1991) 137–177.
[61] J. Kurepa, D. Herouart, M. Van Montagu, D. Inze, Differential expression of CuZn- and Fe-superoxide dismutase genes of tobacco during development, oxidative stress and hormonal treatment, Plant Cell Physiol. 38 (1997) 463–470.
[62] Olsen A, A Study of the Antioxidant Properties of Kinetin in vitro and in vivo, M.Sc. thesis, Department of Molecular and Structural Biology, University of Aarhus, Denmark, 1999.