Inorganic polyphosphate in cardiac myocytes: from bioenergetics to the permeability transition pore and cell survival

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Abstract

Inorganic polyphosphate (polyP) is a linear polymer of P_i residues linked together by high-energy phosphoanhydride bonds as in ATP. PolyP is present in all living organisms ranging from bacteria to human and possibly even predating life of this planet. The length of polyP chain can vary from just a few phosphates to several thousand phosphate units long, depending on the organism and the tissue in which it is synthesized. PolyP was extensively studied in prokaryotes and unicellular eukaryotes by Kulaev's group in the Russian Academy of Sciences and by the Nobel Prize Laureate Arthur Kornberg at Stanford University. Recently, we reported that mitochondria of cardiac ventricular myocytes contain significant amounts (280 ± 60 pmol/mg of protein) of polyP with an average length of 25 P_i and that polyP is involved in Ca^{2+}-dependent activation of the mitochondrial permeability transition pore (mPTP). Enzymatic polyP depletion prevented Ca^{2+}-induced mPTP opening during ischaemia; however, it did not affect reactive oxygen species (ROS)-mediated mPTP opening during reperfusion and even enhanced cell death in cardiac myocytes. We found that ROS generation was actually enhanced in polyP-depleted cells demonstrating that polyP protects cardiac myocytes against enhanced ROS formation. Furthermore, polyP concentration was dynamically changed during activation of the mitochondrial respiratory chain and stress conditions such as ischaemia/reperfusion (I/R) and heart failure (HF) indicating that polyP is required for the normal heart metabolism. This review discusses the current literature on the roles of polyP in cardiovascular health and disease.

Introduction

Inorganic polyphosphate (polyP) is one of the several molecules on earth that effectively store energy within their covalent bonds. Inorganic polyPs are linear polymers of P_i residues linked together by high-energy phosphoanhydride bonds (Figure 1A). These polymers are widely distributed in nature, from archaebacteria, eubacteria, fungi, algae and protozoa to higher plants, animals and humans [1–4]. Neglected and long regarded as a molecular fossil, polyP has a variety of significant functions in bacteria such as a (i) source of energy [2,5], (ii) phosphate reservoir [5], (iii) donor for sugar and adenylate kinases [6], (iv) chelator for divalent cations [7], (v) buffer against alkaline stress [8], (vi) regulator of development [1] and (vii) structural element in competence for DNA entry and transformation [9]. Even though most of polyP research has been performed in microorganisms, the presence of polyP has been demonstrated in many tissues such as rodent liver, kidney, lungs, brain and heart [10], rabbit heart [11,12], osteoblasts [13] as well as in human granulocytes [14], platelets [15,16] and fibroblasts [17]. In striking contrast with microorganisms where polyP is present in millimolar (50–120 mM) concentrations, levels of 25–200 μM (it terms of P_i residues) were found in vast majority of mammalian tissues [10,11]. Interestingly, the total amount of polyP extracted from mouse tissues was almost 2-fold higher compared with that detected in rat samples: with the highest amount detected in the mouse heart (~114 ± 35 μM), brain (95 ± 24 μM), lungs (~91 ± 16 μM) and kidney (64 ± 9 μM) and the lower amounts reported in liver (38 ± 4 μM) [10]. The exceptions are platelets and mast cells which contain millimolar concentrations of polyP in electron dense granules [18]. Intracellular distribution of polyP also varies with relatively higher levels of polyP detected in nuclei (89 ± 7 μM) and plasma membranes (43 ± 3 μM) isolated from rat liver compared with the cytosol (12 ± 2 μM), mitochondria (11 ± 0.6 μM) and microsome fractions (4 ± 0.05 μM) [10]. Also, certain cancer cells (e.g. myeloma plasma cells) accumulate unusually high levels

Key words: heart failure, Inorganic polyphosphate, ischaemia-reperfusion injury, mitochondria, mitochondrial permeability transition pore, poly-β-hydroxybutyrate.

Abbreviations: CsA, cyclosporine A; FCCP, carbonyl cyanide *p*-(tri-fluromethoxy)phenyl-hydrazone; HF, heart failure; I/R, ischaemia/reperfusion; MCU, mitochondrial Ca^{2+} uniporter; MnTBAP, Mn(III)tetrakis (4-benzoic acid) porphyrin; mPTP, mitochondrial permeability transition pore.; PAP, polyP–AMP–phosphotransferases; PHB, poly-β-hydroxybutyrate; polyP, inorganic polyphosphate; PPK, polyP kinase; PPN, endopolyphosphatase; PPX, exopolyphosphatase enzyme; ROS, reactive oxygen species; TMRM, tetramethylrhodamine, methyl ester.

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Inorganic Polyphosphate (polyP) Physiology: Held at Charles Darwin House, London, UK, September 7 2015.

of polyP in the nucleoli which is associated with the enhanced levels of nuclear transcription in these cells [19]. The total level of polyP in U266 myeloma cells was approximately 92 ± 6 pmol/10^6 cells (or 65 μM) compared with 4.5 ± 2 pmol/10^6 cells detected in total human peripheral blood mononuclear cells [19]. It was previously demonstrated by Kornberg and colleagues [20] that polyP stimulates mammalian protein kinase mTOR (mammalian target of rapamycin) involved in the proliferation of human breast carcinoma MCF-7 cells. However, the role of polyP in cancer cells requires further clarification since another study [21] reported that polyP effectively blocks *in vivo* pulmonary metastasis of B16BL6 cells by suppression of neovascularization, whereas it does not affect proliferation or adhesion to extracellular matrix proteins.

The chain length of polyP may range from 3 to more than 1000 P_i residues; it can be analysed on urea/polyacrylamide gels stained with Toluidine Blue or DAPI [22–26]. Our studies performed on mitochondria isolated from rabbit hearts detected the presence of ~200 μM (280 pmol/mg of protein) short-chain polyP with an average chain length of 25 P_is (Figure 1A). Because polyP is found in small amounts in mammalian cells, it does not serve as phosphate or energy storage but is implicated in cell proliferation [20], angiogenesis [21], apoptosis [27], osteoblast function [28], blood clotting and inflammation [15,29–31], cell bioenergetics [12,32], ion channel function [11,33–35] and nuclear transcription [19]. These new discoveries compelled us to take a fresh look at this natural polymer that has been ignored in biochemistry textbooks for a long time.

Sources of polyPs in mammalian cells

PolyPs are also detected in human cells [10,14,17,35,36]; however, the enzyme responsible for polyP generation in mammalian cells has not been identified yet [4]. Human and animals could receive a significant amount of polyP with food and drinking water. PolyPs are legally permitted food additives (E450–452) [37,38] and are widely used to treat fish, fish fillet, shrimp and meat products to improve their water holding capacity, reduce the amount of thaw drip and increase their freezing capacity. PolyP filters are common components of water purifying commercial systems. PolyPs are also used as fertilizers and flame retardants due to their unique properties, inexpensiveness, non-toxicity and biodegradability. Furthermore, it has been discovered that human gastrointestinal tract bacteria (probiotics) produce polyP and that polyP is responsible for probiotic actions that protect the intestinal epithelia from oxidant stress and also improve epithelial injury due to excess inflammation [39]. Intriguingly, recent studies [40,41] demonstrated that probiotic administration attenuates myocardial infarction following ischaemia/reperfusion (I/R) injury and myocardial hypertrophy and heart failure (HF) following myocardial infarction in the rat. PolyP levels were not measured in these studies; however, it is plausible to speculate that polyP produced by gut microbiota could also exert a cardioprotective role for the host organism.

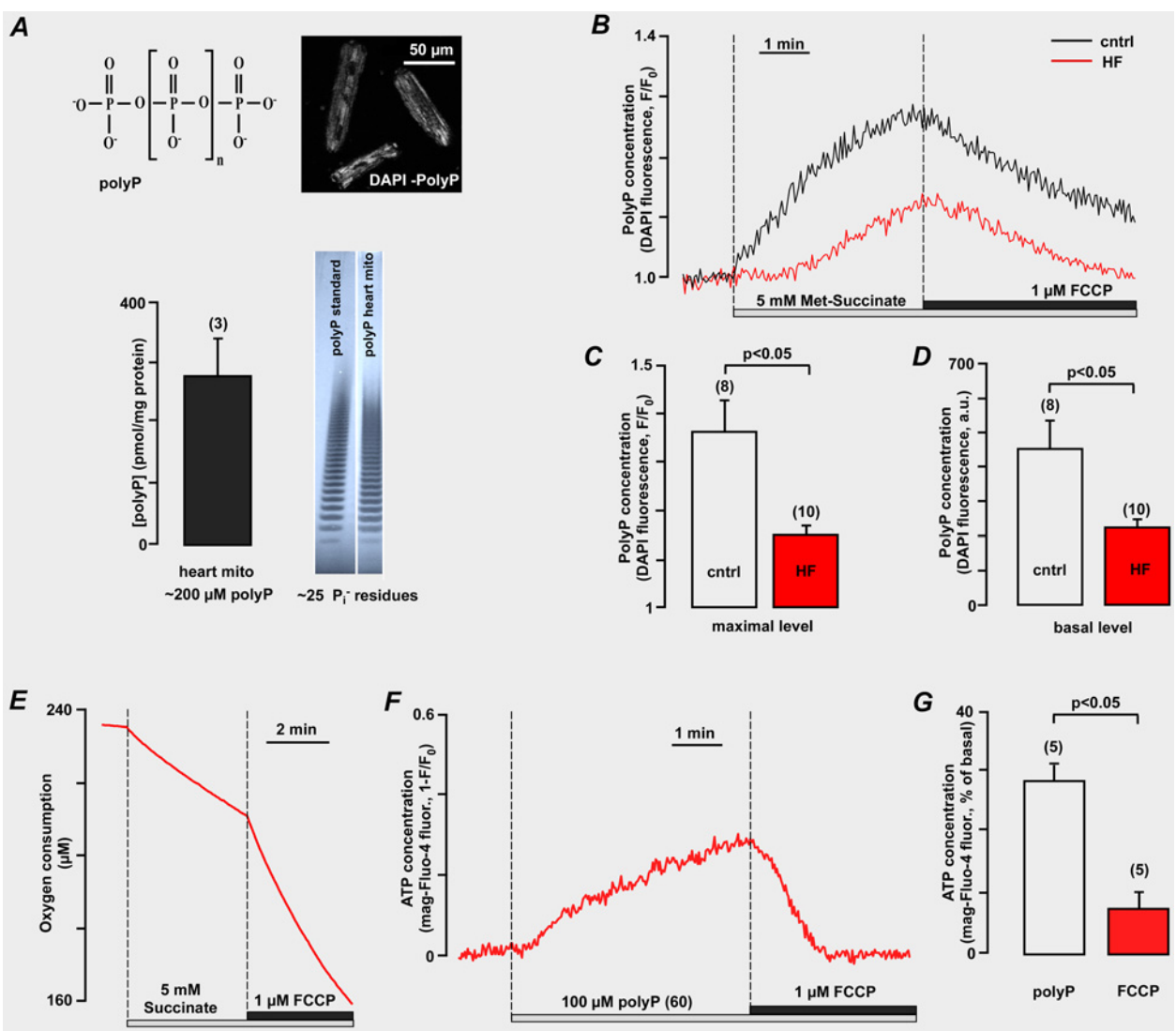
In prokaryotes, polyP is synthesized enzymatically by polyP kinase 1 (PPK1) via transferring the terminal phosphate from ATP to the end of the growing polyP chain and this reaction is fully reversible and may allow the bacteria to synthesize ATP from stored polyP in times of starvation and environmental stress [42,43]. Null mutants of PPK1, with low polyP levels, are deficient in survival: namely, they show deficient responses to physical–chemical stresses and predation [42,43]. So far, no PPK1 homologue has been identified in higher-order eukaryotes even though it is structurally similar to phospholipase D [44] and, therefore, PPK1 exhibits potential as a novel target for chemotherapy that would affect both virulence and susceptibility to anti-bacterial compounds [43]. Moreover, it has been demonstrated that plasma membrane Ca^{2+}-ATPase from human erythrocytes may function as a PPK, i.e. it exhibits ATP-polyP transferase and polyP-ADP transferase activities [45]. The mitochondrial F_1F_0-ATP synthase can also contribute to polyP generation (see below and [12,32] for details). Two bacterial enzymes [the second PPK (PPK2) and polyP–AMP–phosphotransferases (PAP)] use polyP as a substrate. PPK2 actually resembles mammalian thymidylate kinase [46]. PAP uses polyP as a substrate to phosphorylate AMP to ADP, an immediate precursor of ATP. PolyP is degraded by both endopolyphosphatases (PPNs) and exopolyphosphatases (PPXs). In mammalians, a long-chain PPN was purified from rat and bovine brain [47], a human metastasis regulator protein H-prune was identified as a short-chain specific PPX [48] and mammalian intestinal alkaline phosphatase was characterized as a very active PPX [49]. In addition, 41 % identity has been found between yeast PPX PP1 gene product and human acid sphingomyelinase-like phosphodiesterase [50,51]. In the colon, this enzyme may play anti-proliferative and anti-inflammatory roles via ceramide generation, reducing the lysophosphatidic acid formation and inactivating the platelet-activating factor and mutations in its gene have been found in cancer cells of the intestines [50]. Interestingly, the human protein H-prune exhibits 91 % identity with the sequences of yeast PPX1 [51].

Mitochondrial metabolism of polyP in mammalian cells

At present, very little is known about the molecular details of polyP metabolism in mammalian cells; however, it has been demonstrated that newly identified human mitochondrial NAD kinase utilizes not only ATP but also polyP as the phosphoryl donor [52]. NAD kinase is the sole $NADP^+$-biosynthetic enzyme known to catalyse phosphorylation of NAD^+ to yield $NADP^+$ and plays a role in the defence against mitochondrial oxidative stress [53]. To date, no mammalian polyP producing enzymes have been identified; however, it has been demonstrated that polyP production in mammalian cells depends on the metabolic state of the

Figure 1 | **Mitochondrial polyP detection in healthy and HF cardiac myocytes**

(**A**) The upper panel shows the structure of inorganic polyP. The *n* represents the number of phosphate residues in the polyP chain. It could vary from ten to hundreds of units. The right panel image demonstrates polyP detection in freshly isolated rabbit ventricular myocytes using DAPI as a sensor for polyP (λ_{ex} = 408 nm, λ_{em} = 552–617 nm). The bottom panel shows the average amount of polyP in rabbit heart mitochondria (left) and gel images of polyP standard and polyP sample from isolated rabbit mitochondria (right). (**B**) Original recordings of DAPI fluorescence changes in intact cardiac myocytes stimulated with 5 mM methyl-succinate followed by 1 μM FCCP from control (black) and failing myocytes (red). DAPI fluorescence represents changes in polyP concentration. (**C**) Average values of maximal DAPI fluorescence after methyl-succinate addition in control (black) and HF (red) cells. (**D**): Average values of basal DAPI fluorescence in control (black) and HF (red) myocytes. (**E**) Recording of mitochondrial oxygen consumption in permeabilized rabbit ventricular myocytes upon stimulation of the mitochondrial respiratory chain with 5 mM succinate and subsequent addition of 1 μM FCCP. (**F**) Recording of mitochondrial ATP generation in permeabilized rabbit ventricular myocytes upon addition of 100 μM polyP (with 60 phosphate units) and subsequent addition of 1 μM FCCP. (**G**) Summary of the mitochondrial ATP generation in permeabilized rabbit ventricular myocytes. Modified with permission from [11,12,54]: Seidlmayer, L.K., Gomez-Garcia, M.R., Blatter, L.A., Pavlov, E. and Dedkova, E.N. (2012) Inorganic polyphosphate is a potent activator of the mitochondrial permeability transition pore in cardiac myocytes. J. Gen. Physiol. 139, 321–331; Seidlmayer, L.K., Blatter, L.A., Pavlov, E. and Dedkova, E.N. (2012) Inorganic polyphosphate-an unusual suspect of the mitochondrial permeability transition mystery. Channels 6, 463–467; Seidlmayer, L.K., Juettner, V.V., Kettlewell, S., Pavlov, E.V., Blatter, L.A. and Dedkova, E.N. (2015) Distinct mPTP activation mechanisms in ischaemia-reperfusion: contributions of Ca^{2+}, ROS, pH, and inorganic polyphosphate. Cardiovasc. Res. 106, 237–248.



mitochondria [12,32]. Experiments performed on isolated rat liver mitochondria, cultured intact cells [astrocytes, human embryonic kidney (HEK)293; 32] and rabbit cardiomyocytes [12,54] demonstrated that levels of polyP were increased by substrates of the mitochondrial respiratory chain and in turn reduced by mitochondrial inhibitor (rotenone) or respiratory chain uncoupler carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP). Oligomycin, an inhibitor of mitochondrial F_1F_0-ATP-synthase, blocked the production of polyP. These data suggest that in mammalian cells mitochondrial polyP production is closely related to the activity of the oligomycin-dependent F_1F_0-ATP synthase. However, whether or not F_1F_0-ATP synthase is polyP generating enzyme remains to be validated.

We investigated the kinetics of mitochondrial polyP metabolism [12] in intact ventricular cardiomyocytes isolated from control rabbits and animals with HF (combined aortic insufficiency and stenosis model) [55]. The relative changes in levels of polyP were measured using the fluorescent probe DAPI, with a protocol optimized specifically for polyP detection [11,12,56]. As demonstrated in Figure 1(B) addition of membrane permeable methyl-succinate, the substrate of the complex II of the mitochondrial respiratory chain, resulted in an increase in DAPI fluorescence by $36 \pm 8\%$ ($n = 8$), indicating significant stimulation of the production of mitochondrial polyP (Figure 1C). On the other hand, uncoupling of respiration with FCCP decreased DAPI fluorescence by $29 \pm 4\%$ ($n = 8$) presumably due to the stimulation of polyP hydrolysis. This indicates that polyP concentration in cardiac myocytes is variable and depends on levels of energy substrates and the degree of coupling of the mitochondrial respiratory chain. Moreover, we found that polyP metabolism was significantly suppressed in mitochondria of HF myocytes. Addition of methyl-succinate caused only a moderate increase in DAPI fluorescence ($16 \pm 2\%$, $n = 10$; Figures 1B and 1C) [12]. Also, the basal polyP levels were significantly lower in conditions of HF (224 ± 21 a.u. (arbitrary units) in HF compared with 453 ± 80 in control; Figure 1D) [4]. We performed these experiments in the presence of phosphate but in the absence of ADP. Under these conditions, the mitochondrial respiratory chain is active, but its activity is not coupled to ATP production [57–59]. In a separate set of experiments, we monitored mitochondrial respiration in permeabilized rabbit ventricular myocytes [55] and demonstrated that addition of 5 mM succinate induced a 2.9 ± 4-fold increase in the rate of respiration. This rate was $42 \pm 2\%$ ($n = 6$) of the maximal rate of mitochondrial respiration achieved in the presence of 1 μM FCCP (Figure 1E). Thus, the level of polyP in mitochondria is dependent on the activity of mitochondrial respiration rather than the production of ATP. These results suggest that mitochondria do not use ATP as a substrate for polyP synthesis. In fact, using mag-Fluo-4 as indirect indicator for ATP generation [57], we were able to demonstrate that addition of 100 μM external synthetic polyP (with a chain length of 60 phosphates, a gift from Dr T. Shiba, Regenetiss Inc., Japan) to permeabilized rabbit ventricular myocytes induced a significant increase in mitochondrial ATP levels (Figures 1F and 1G). Addition of mitochondrial uncoupler FCCP decreased ATP levels back to basal level indicating that this ATP generation was dependent on the activity of the mitochondrial respiratory chain (Figure 1G).

The phosphate groups in polyPs are linked in the same way that they are in ATP and ADP; this chemical equivalence means that phosphoryl transfer from polyP to ADP to make ATP is a relatively simple shift between similar materials, with essentially no by-products: ADP + $(polyP)_n \iff$ ATP + $(polyP)_{n-1}$ [3]. It has been suggested that the sequestered polyP serves as a storage form of energy which could be mobilized to rephosphorylate ADP to ATP via the PPK reaction in prokaryotes [3]. Our data indicate that a similar mechanism takes place in cardiac myocytes and one of the enzymes of the mitochondrial respiratory chain (possibly mitochondrial F_1F_0-ATP-synthase as discussed above) facilitates this reaction. Although cells use ATP to drive synthetic reactions, ATP is not a primary energy source [60], but rather is an energy transfer molecule that picks up energy from an energy source and then delivers it to energy-requiring reactions. The chemical energy content of ATP is present in the pyrophosphate bonds that link the second and third phosphate groups of ATP. These are anhydride bonds and their chemical energy is released by energetically downhill group transfer reactions of the phosphate group to other molecules, an activating process called phosphorylation.

Interestingly, when intact ventricular myocytes were exposed to simulated I/R, we found that a slow increase in polyP synthesis during ischaemia was followed by a significant increase in polyP generation on reperfusion (Figures 2A and 2B). As we demonstrated before [54], these conditions of simulated I/R were accompanied by a significant increase in reactive oxygen species (ROS) generation (Figures 2C and 2D). Using MitoSOX Red as sensor for superoxide (O_2^-), we detected increase in O_2^- generation already during ischaemia with only small additional increase in fluorescence observed during reperfusion (Figures 2C and 2D). In both control and polyP-depleted cells, the fluorescence increase was significantly attenuated by MnTBAP, a cell-permeable superoxide dismutase mimetic (Figure 2D), confirming the fidelity of O_2^- detection with MitoSOX Red. Blocking mitochondrial Ca^{2+} uptake through mitochondrial Ca^{2+} uniporter (MCU) with 1 μM Ru360 completely prevented ROS generation during I/R in both control and polyP-depleted (Figures 2C and 2D) cells. This indicates that Ca^{2+} entering via MCU stimulates mitochondrial O_2^- generation during ischaemia. The mitochondrial respiratory chain appeared to be the main source of O_2^- generation since exposure to a 'mock' ischaemia solution lacking sodium cyanide prevented the increase in MitoSOX Red fluorescence (Figure 2D). Moreover, we found that during ischaemia either polyP depletion or cyclosporine A [CsA, inhibits mitochondrial permeability transition pore (mPTP)] treatment led to an increased mitochondrial O_2^- accumulation (Figures 2C and

Figure 2 | **Measurement of polyP and ROS levels in control and poly-depleted myocytes during I/R**

(**A**) Original recordings of DAPI fluorescence in control (with overexpressed GFP) and polyP-depleted (PPX) cardiac myocytes exposed to 20 min of simulated chemical ischaemia followed by a 15 min reperfusion period in normal Tyrode solution. (**B**) Average values of maximal DAPI fluorescence at the end of 20 min ischaemia and 15 min reperfusion in control rabbit ventricular cells. (**C**) Superoxide generation measured with MitoSox Red during I/R in control and polyP-depleted (PPX) cells in the absence and presence of 1 μM Ru360 (inhibits mitochondrial calcium uniporter) and 1 μM CsA (inhibits mPTP). (**D**) Average amplitude of MitoSox Red fluorescence changes at the end of ischaemia and at the end of reperfusion in control and polyP-depleted cells. Superoxide scavenger (50 μM MnTBAP, [5,10,15,20-tetrakis(4-carboxyphenyl)-21H,23H-porphine]manganese(III)chloride) was used to verify the fidelity of MitoSox Red for superoxide. 'Mock' ischaemia solution did not contain NaCN. Modified with permission from [54]: Seidlmayer, L.K., Juettner, V.V., Kettlewell, S., Pavlov, E.V., Blatter, L.A. and Dedkova, E.N. (2015) Distinct mPTP activation mechanisms in ischaemia-reperfusion: contributions of Ca^{2+}, ROS, pH, and inorganic polyphosphate. Cardiovasc. Res. 106, 237–248.

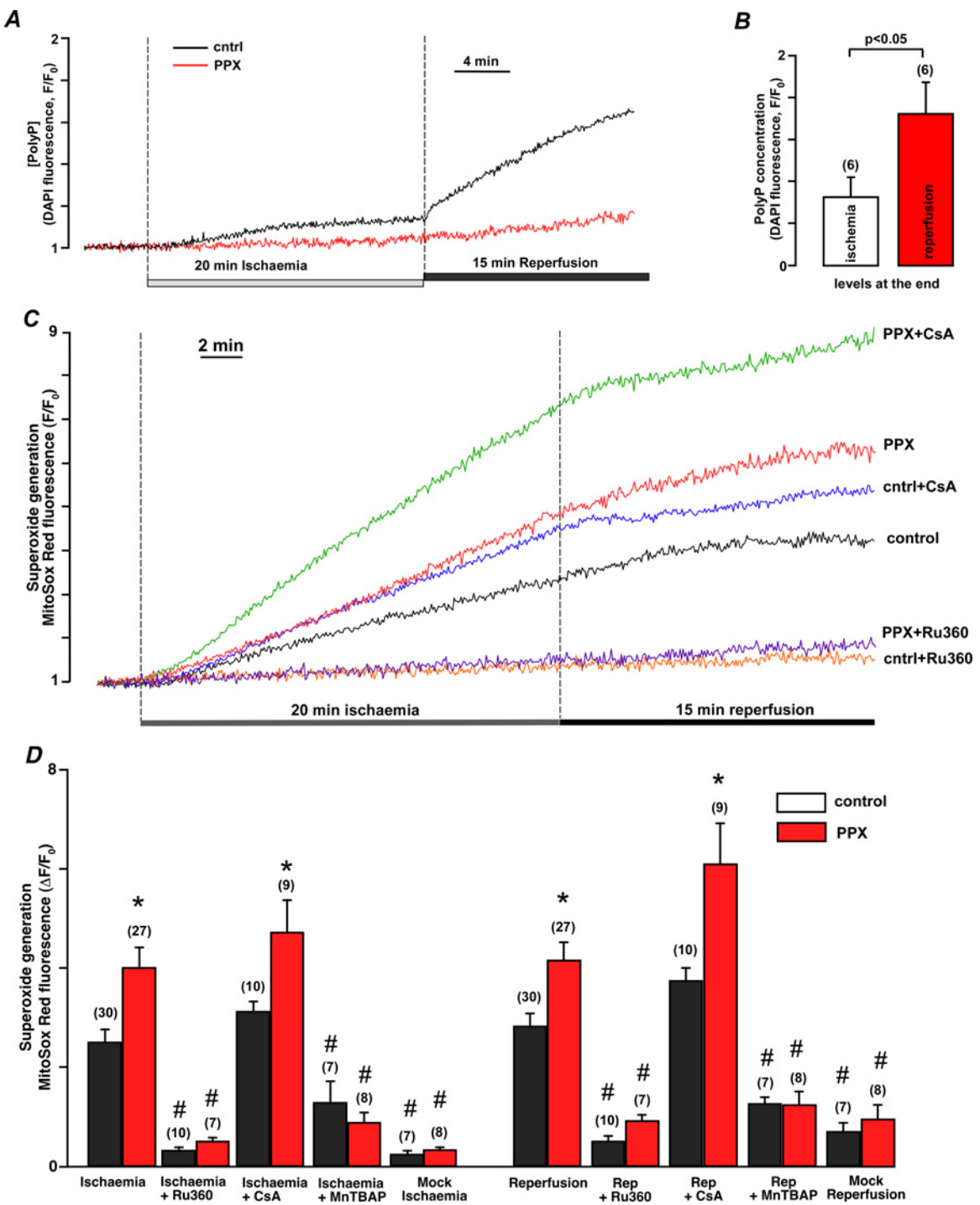
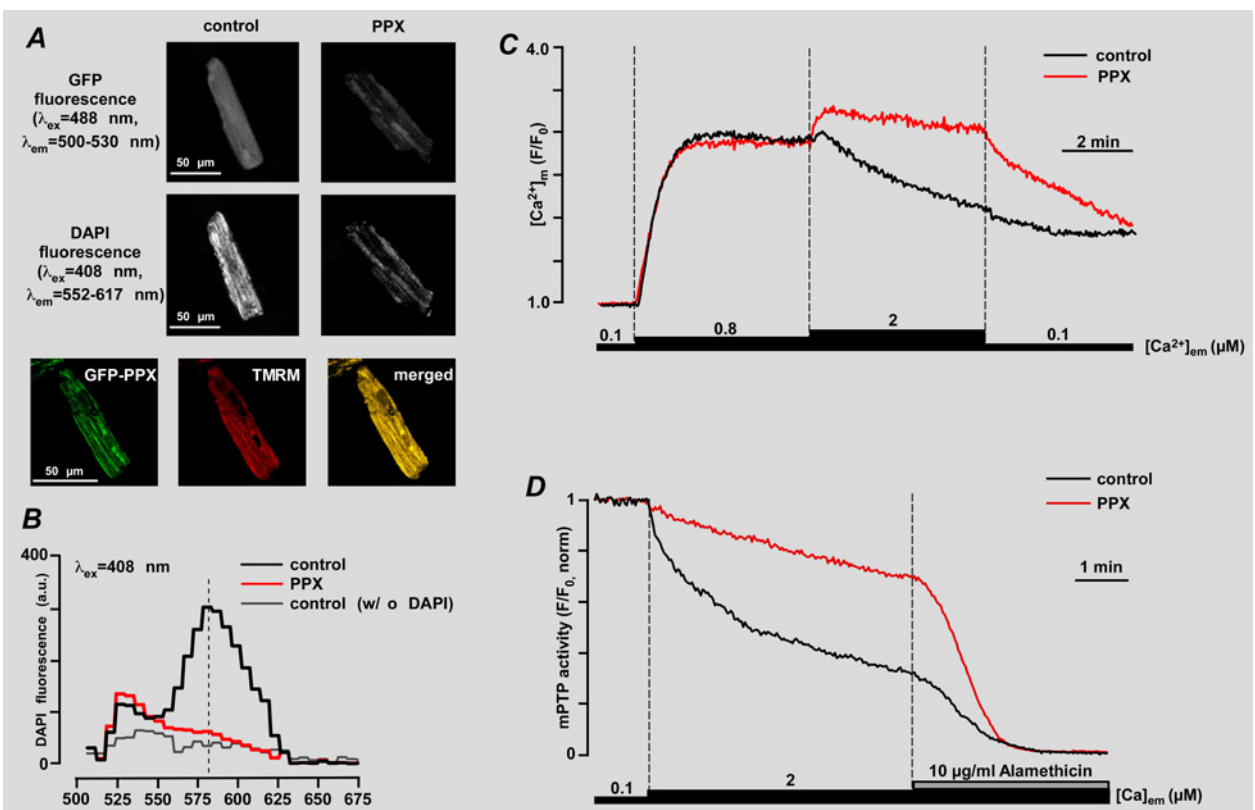


Figure 3 | PolyP depletion prevents opening of the permeability transition pore induced by mitochondrial Ca^{2+} overload

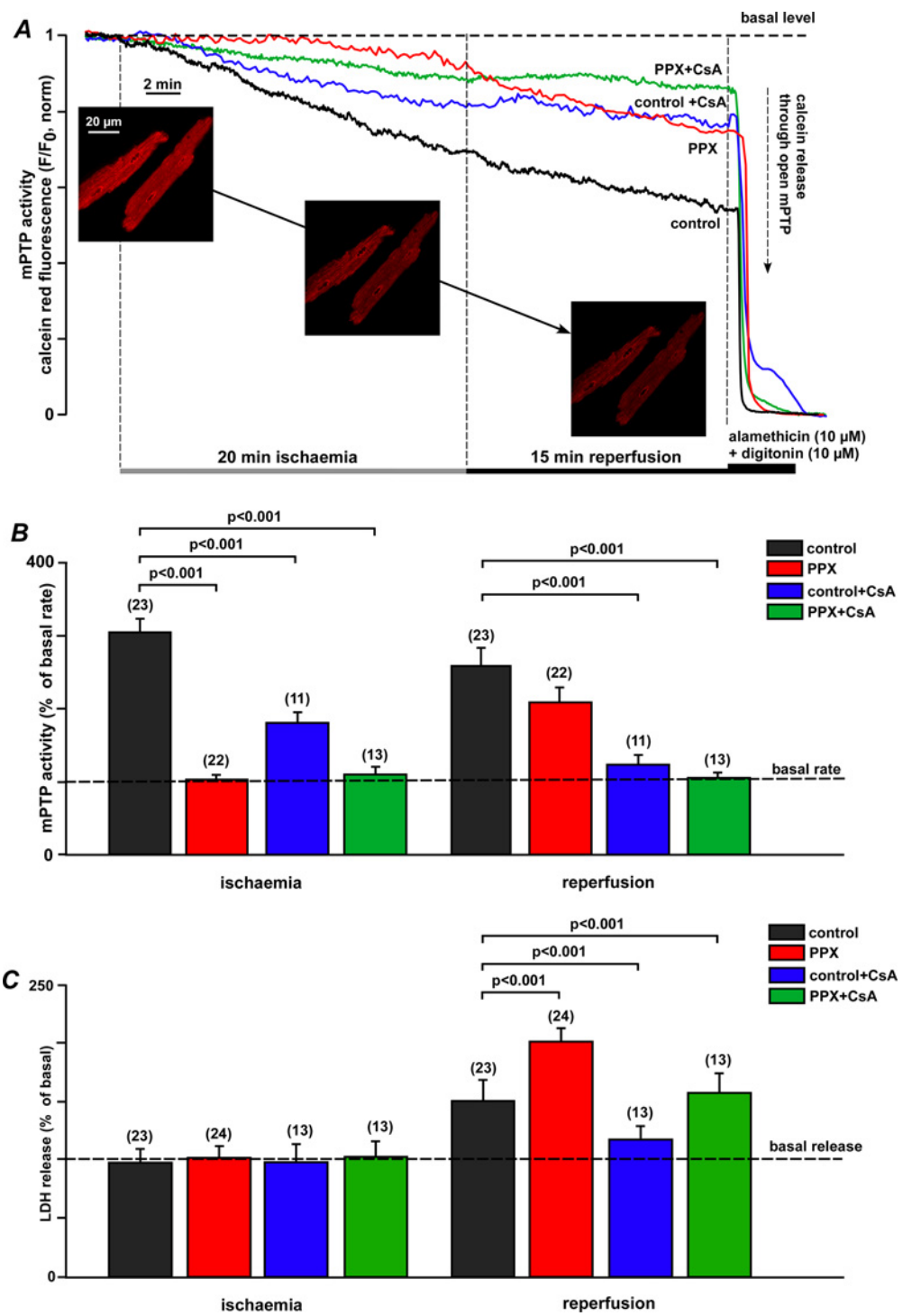
(**A**) Images of control GFP (left) and PPX expressing cardiac myocytes (right). The upper panel shows global GFP fluorescence at 500–530 nm that reveals the mitochondrial fluorescence pattern in PPX expressing cells and a homogeneously distributed fluorescence in control cells. The middle panel shows the decrease in DAPI fluorescence in polyP-depleted cells. The bottom panel shows co-localization of GFP–PPX signal with mitochondria. Mitochondrial membrane potential sensitive dye tetramethylrhodamine, methyl ester (TMRM) was used as a mitochondrial signal and the degree of overlay is presented in shades of yellow in the merged image. The bottom panel shows the decrease in DAPI fluorescence in polyP-depleted cells. (**B**) Fluorescence spectrum of DAPI (5 μM) loaded myocytes expressing control GFP (black), PPX (red) and control GFP cells not loaded with DAPI (grey). (**C**) Original recordings of mitochondrial Ca^{2+} concentration ($[Ca^{2+}]_m$ in permeabilized X-Rhod-1-loaded cells upon stepwise elevation of $[Ca^{2+}]_{em}$ from 0.1 to 0.8 to 2 μM and subsequent return to 0.1 μM in control (black) an polyP-depleted (red) cells. (**D**) Original recordings of mPTP opening using Calcein Red release from mitochondria of permeabilized control (black) and polyP-depleted (PPX expressing, red) myocytes. After permeabilization, cells were exposed to 2 μM Ca^{2+} and 10 μg/ml alamethicin was added at the end of the experiment to achieve the maximal Calcein Red release from mitochondria. Modified with permission from [11]: Seidlmayer, L.K., Gomez-Garcia, M.R., Blatter, L.A., Pavlov, E. and Dedkova, E.N. (2012) Inorganic polyphosphate is a potent activator of the mitochondrial permeability transition pore in cardiac myocytes. J. Gen. Physiol. 139, 321–331.



2D). Taking into account that this effect was further enhanced in polyP-depleted cells in the presence of CsA, (Figures 2C and 2D) it is likely that polyP regulation of O_2^- production is not directly linked to its ability to inhibit Ca^{2+}-induced mPTP. Together, these data indicate that mitochondrial Ca^{2+} uptake through MCU was stimulating O_2^- production within mitochondrial matrix. Moreover, we found that polyP was protecting mitochondria from the excessive O_2^- generation. This observation raises the intriguing possibility that, similar to bacteria, mammalian cells could produce polyP in response to cell stress and that diminished polyP synthesis observed in HF myocytes results from the complex remodelling processes during cardiac hypertrophy and HF. A recent study [61] determined that in *Escherichia coli* bacteria polyP acts as an efficient protein chaperon which stabilizes proteins *in vivo*, diminishes the need for other chaperone systems to survive proteotoxic stress (temperature, low pH, oxidants) conditions and protects a wide variety of proteins against stress-induced unfolding and aggregation. It has been demonstrated that wild-type *E. coli* strains generated significant amounts of polyP in response to oxidative stress. The finding that polyP has stress-protective chaperone activities that resemble the activity of small heat shock proteins is very exciting; however, additional research is

Figure 4 | Effects of polyP depletion and CsA on mPTP activity and necrotic cell death following I/R

(**A**) Normalized traces of Calcein Red fluorescence changes during 20-min ischaemia followed by 15 min of reperfusion in control (black) and polyP-depleted (red) cells in the absence or presence of 1 μM of CsA (mPTP inhibitor). Inserts show images of Calcein-loaded cardiomyocytes before and after exposure to I/R. (**B**) Summary of Calcein Red release from mitochondria (as percent of basal rate) at the end of ischaemia and reperfusion. (**C**) Cell death [percentage of lactate dehydrogenase (LDH) release with respect to basal LDH release rates] measured at the end of ischaemia and reperfusion in control and polyP-depleted (PPX) cells in the absence or presence of 1 μM CsA. *n* is the number of hearts used in each experiment. Modified with permission from [54]: Seidlmayer, L.K., Juettner, V.V., Kettlewell, S., Pavlov, E.V., Blatter, L.A. and Dedkova, E.N. (2015) Distinct mPTP activation mechanisms in ischaemia-reperfusion: contributions of Ca^{2+}, ROS, pH, and inorganic polyphosphate. Cardiovasc. Res. 106, 237–248.



required to determine the mechanisms of protein aggregation prevention by polyP and the protein targets of polyP in mammalian cells.

Inorganic polyP as a calcium sensor for the mitochondrial permeability transition pore

One of the most intriguing and least intuitive roles of polyP is its involvement in membrane ion transport. In 1988, Reusch and Sadoff [9], using bilayer techniques, demonstrated that genetically competent *E. coli* bacteria contain an ion channel formed by a complex of polyP and poly-β-hydroxybutyrate (PHB) [9]. The channel formed by polyP–Ca^{2+}–PHB interaction was selective for cations with a preference for Ca^{2+} [9,62]. Later a similar polyP–Ca^{2+}–PHB channel was isolated from rat liver mitochondria [63]. Interestingly, in addition to the cation selective conductance state this mitochondrial complex also demonstrated a high-conductance, weakly-selective, voltage-dependent state. These properties in many ways reflected the behaviour of the mPTP as seen in patch-clamp studies of native mitochondrial membranes [64,65]. Interestingly, the polyP–Ca^{2+}–PHB channel of bacterial origin also has this high conductance state [66] and the transition of the channel into a high conductance state would most probably be deleterious for bacterial organisms, raising the question whether most of the time the bacterial channel is either closed or is in the low conductance cationic state. The different bacterial conductance states are reminiscent of conductance states proposed for the mPTP [67–69]. The parallels between bacteria and mitochondria also suggest that similar cationic channels may play a role in normal mitochondrial function. In support of such notion the polyP–Ca^{2+}–PHB complex has been detected in various eukaryotic organisms and cellular compartments suggesting a potential physiological role [70]. Currently, the direct test whether a polyP–Ca^{2+}–PHB complex indeed forms the pore part of the mPTP in intact mitochondria remains an experimental challenge. Nonetheless, the idea that the presence of polyP in intact mitochondria is an essential condition for mPTP opening remains an intriguing hypothesis. Indeed, it was shown that mitochondria of cultured cells with reduced levels of polyP are more resistant towards Ca^{2+}-induced mPTP opening [35]. We demonstrated that enzymatic depletion of polyP from cells achieved by overexpression of the mitochondria-targeted yeast PPX (MTS–GFP–scPPX1; Figures 3A and 3B) resulted in decreased openings of Ca^{2+}-induced mPTP in permeabilized rabbit ventricular cardiomyocytes. In contrast with non-excitable cells [35], polyP depletion did not affect the ability of mitochondria to accumulate Ca^{2+} (Figure 3C); however, significantly decreased Ca^{2+}-induced openings of mPTP (Figure 3D) and prevented Ca^{2+}-induced loss of mitochondrial membrane potential (result not shown) indicating that polyP is a potent activator of Ca^{2+}-induced mPTP. On the other hand, when mPTP activity was monitored in conditions of simulated I/R accompanied by massive ROS generation, polyP depletion was not able to prevent mPTP opening and cell death. In fact, as we demonstrated earlier [54], ROS generation and cell death was significantly increased under conditions of I/R in polyP-depleted cells. We found different modes in mPTP activity during ischaemia and reperfusion and that these modes were affected differently by polyP. In agreement with our data obtained on permeabilized cells [11], polyP depletion prevented Ca^{2+}-induced low conductance mPTP mode observed during ischaemia, however it did not affect ROS-mediated mPTP opening in the high-conductance mode during reperfusion (Figures 4A and 4B). Interestingly, polyP-mediated mPTP opening during ischaemia was not associated with cell death (Figure 4C), whereas ROS-mediated mPTP opening during reperfusion was associated with increased cell death. Furthermore, cell death during reperfusion was significantly enhanced in polyP-depleted cells (Figure 4C). These exciting findings indicate that polyP has a dual effect on mPTP activity, promoting the transient opening of Ca^{2+}-induced mPTP opening which can prevent mitochondria from Ca^{2+} overload. On the other hand, polyP was required for protection against oxidative stress-induced mPTP opening and cell death. It is unclear at this point, whether this effect of polyP was related to the recently discovered chaperone activity of polyP or the direct effect of polyP on mPTP. Recent data suggest that dimers of the F_1F_0-ATP synthase can form channels with characteristics similar to the mPTP [71]; however, the molecular details of channel formation by F_1F_0-ATP synthase remain unclear. Particular attention has been brought to the subunit *c* of the F_1F_0-ATP synthase as a potential component of the mPTP [72,73]. Interestingly, an interaction of polyP–Ca^{2+}–PHB complex with subunit c of F_1F_0-ATP synthase was reported back in 2005 [63] and therefore it is possible that polyP could provide a fine tuning of mPTP regulation or actually mediate Ca^{2+} transfer through mPTP. The physiological importance of transient (low-conductance) mPTP opening, which does not lead to cell death [74], has been suggested to mediate ischaemic pre-conditioning-induced protection [75,76] via (i) regulation of the mitochondrial matrix Ca^{2+} concentration [68]; (ii) induction of mild mitochondrial uncoupling [77]; and (iii) regulation of mitochondrial ROS release [78]. We now suggest that inorganic polyP also contributes to the mPTP opening in a low-conductance mode. Furthermore, our findings show that depletion of polyP was associated with enhanced cell death on reperfusion, indicating that stimulation of polyP production rather than inhibition of Ca^{2+} uptake on reperfusion could be beneficial for cardioprotection.

Concluding remarks

The ubiquity of polyP and the variation in its chain length, location and metabolism indicate the relevant functions of this polymer, including those in animal systems. The current data reviewed here are consistent with a key role for polyP in activation of the mPTP and in regulation of stress-induced cell death in cardiac myocytes. We speculate

that endogenous levels of mitochondrial polyP reflect the ability of the cell to survive stress conditions. Mitochondrial polyP concentration is subject to remodelling processes in HF and during I/R. In this sense, a more comprehensive understanding of polyP biochemistry during cardiovascular stress may unravel additional targets that could be effective in the controlling mPTP activity and shed new light on polyP metabolism.

Funding

This work was supported by the American Heart Association [grant-number 15GRNT25090220] to E.N.D.

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Received 26 November 2015
doi:10.1042/BST20150218