Plant & Cell Physiol. 23(1): 137–146 (1982)

Phosphorylation of Chromosomal Proteins in Resting and Wounded Potato Tuber Tissues

Willi Schäfer and Günter Kahl

Department of Biology, University of Frankfurt, 6000 Frankfurt/Main, Fed. Rep. Germany

Wounding of quiescent white potato tuber tissue enhances chromatin-bound protein phosphokinase activity, which exhibits two distinct phases during wound-healing. A moderate activation of the enzymes up to 20 hr after injury is followed by a dramatic increase in activity with a peak at 50 hr. This time-course resembles that of chromatin-bound DNA-dependent RNA polymerase with a peak in activity at about 48 hr after wounding. The kinases phosphorylate endogenous proteins as well as added histones, phosvitin and casein. The incorporated phosphate is stable under standard assay conditions, indicating the absence of protein phosphatases. Sensitivity of the incorporated phosphate toward trypsin and alkali, but not DNase, RNase, hydroxylamine or succinic acid points to seryl- and threonyl-bonds and proteins as acceptor molecules. Kinases from resting tissues are only weakly stimulated even by 100 mM $MgCl_2$, those from wounded tissues exhibit pronounced Mg^{++}-optima at 5–10 mM with endogenous proteins, phosvitin and casein and 50 mM $MgCl_2$ with histones. Wounding also increases the sensitivity of the kinases toward *p*-hydroxymercuribenzoate.

Chromatin preparations from both resting and wounded tissues contain about 40 protein bands after polyacrylamide disc gel electrophoresis. In vitro phosphorylation of these proteins in chromatin from quiescent tissues is comparably low and uniform. Wounding induces changes in the protein and phosphorylation pattern with a general enhancement of phosphorylative capacity and preferential phosphorylation of low molecular weight proteins.

Key words: Non-histone chromosomal proteins — Phosphorylation — Protein phosphokinase — *Solanum tuberosum* L. — Wounding.

Wounding of plant tissues induces a variety of biochemical reactions which ultimately lead to the closure of the wound and thereby to the survival of the organism. In model systems such as white potato tuber tissue, nearly all these reactions are dependent on prior transcription. The rate of transcription itself is very low in intact organs, but rises rapidly after wounding. Template availability increases, DNA-dependent RNA polymerases I and II are activated (Kahl and Wielgat 1976, Kahl and Wechselberger 1977, Wielgat and Kahl 1979) and consequently ribosomal, transfer and messenger RNA are more rapidly synthesized (Kahl 1971). Concomitantly the nucleus and nucleolus increase in volume, nucleolar vacuoles are formed and chromatin material is exported from the nucleolus to the nucleoplasm (Kahl 1973).

The mechanism of gene activation after wounding is not known. However, there is some evidence that nonhistone chromosomal proteins are involved. It has been shown in an in vitro transcription system using potato DNA, potato histones and *E. coli* RNA polymerase that histone-inhibition of RNA synthesis cannot be reverted by nonhistone proteins from resting potato tuber tissues. Non-histone proteins from wounded tissues, on the contrary, are capable of releasing histone-inhibition completely (Kahl et al. 1979). This wound-induced change in the quality

of nonhistone proteins may be brought about by chemical modifications of these proteins such as phosphorylation (Rubin and Rosen 1975, Trewavas 1975). Nonhistone phosphoproteins have been shown to displace histones from distinct sites of DNA in chromatin, thereby releasing genes for transcription (Patel and Thomas 1973, Stein et al. 1975). The interaction of these proteins with DNA depends on their phosphorylation level (Kleinsmith 1973), dephosphorylated nonhistone proteins are not or less active in stimulating specific transcription (Kleinsmith 1975a, Kleinsmith et al. 1976, Spelsberg et al. 1975). Phosphorylation of nonhistone chromosomal proteins may then represent a key process in the activation of genes, at least in animal systems.

The present experiments were aimed at answering two questions. First, is there any quantitative and qualitative change in chromatin-bound phosphokinase activity after wounding? Second, is there a change in the phosphorylation pattern of chromosomal proteins after wounding?

Materials and Methods

Plant material—The plant material and its treatment has been described in detail by Wielgat and Kahl (1979).

Chromatin isolation—Chromatin was isolated essentially as described (Wielgat and Kahl 1979), except that the washed chromatin was layered on top of 10 ml of 1.2 M sucrose in 0.01 M Tris-HCl (pH 8.0) and 0.01 M 2-mercaptoethanol. The gradient was centrifuged for 1.5 hr at 78,000 $\times g$ (Beckman L5 65B, SW 40 rotor) and the chromatin pellet resuspended in 0.01 M Tris-HCl, pH 8.0. Aliquots of this suspension were used for protein phosphokinase and RNA polymerase assays. Protein was determined by the method of Lowry et al. (1951).

Chromatin-bound RNA polymerase and DNA polymerase—RNA polymerase activity was assayed as previously described (Wielgat and Kahl 1979). DNA polymerase activity was estimated in a reaction mixture containing 100 mM Tris-HCl, pH 8.0, bovine serum albumin (0.2 mg/ml), 2.5 mM dithioerythritol, 10 mM KCl, 10 mM $MgCl_2$, 2% glycerol, 0.1 mM each of dATP, dGTP, dCTP and 2 μCi [^{3}H]-dTTP (40–60 Ci/mmol, The Radiochemical Center, Amersham). The reaction was started by the addition of 25 μg chromatin DNA and incubation was at 37°C for 20 min, after which time 5 ml 10% (w/v) trichloroacetic acid containing 0.1 M sodium pyrophosphate was added. The precipitate was collected on glass fiber filters, washed three times with 5% trichloroacetic acid/0.1 M sodium pyrophosphate, dried and the radioactivity on the filters counted by liquid scintillation techniques.

Chromatin-bound protein phosphokinases—Phosphokinase activity with endogenous proteins as substrates was determined in a total volume of 0.25 ml containing 250 μg chromatin protein and 100 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$ and 1 μCi [γ-^{32}P]-ATP (3.2 Ci/mmol, The Radiochemical Center, Amersham). The reaction mixture was incubated for 30 min at 37°C and the reaction was stopped with 5 ml of cold 10% (w/v) trichloroacetic acid. After 30 min on ice each sample was processed as described for RNA polymerase. Corrections were made for nonspecific adsorption of [^{32}P]-ATP to chromatin material. Phosphokinase activity with exogenous proteins as substrates was estimated by adding 50 μg each of calf thymus histones, phosvitin or casein (Sigma) respectively.

Stability of the incorporated phosphate—To test the stability of the incorporated phosphate against nucleases, proteases and NaOH, the chromatin pellet was suspended in 50 mM Tris-buffer, pH 7.5 and incubated with [γ-^{32}P]-ATP for 30 min at 37°C in a scaled-up reaction mixture. The reaction was stopped by boiling for 5 min. Then triplicates were removed and tested for total ^{32}P incorporated into trichloroacetic acid-precipitable material (control). The remaining solution was divided and one part was treated with 50 μg/ml RNase A (Sigma) for 4 hr at 37°C, the second one was made 10 mM with respect to $MgCl_2$ and DNase I (Boehringer, Mannheim) in a final concentration of 40 μg/ml was added and incubated for 15 hr at 37°C. The third part

was incubated with 100 μg/ml trypsin for 24 hr at 37°C and the fourth one brought to 1 N NaOH and placed at 100°C for 20 min. Corrections were made in the controls for volume changes.

For a test of the stability of the incorporated phosphate against NH_2OH and succinic acid the chromatin pellet was dissolved in 50 mM sodium acetate buffer, pH 5.4 and incubated for 30 min at 37°C together with [γ-^{32}P]-ATP. Again the reaction was stopped at 100°C for 5 min and triplicates assayed for total ^{32}P incorporated into TCA-precipitable material (control). The remainder was divided into two parts, one of which was brought to 0.8 M NH_2OH and incubated at 25°C for 10 min, the other of which was treated with 1 M succinic acid at 37°C for 30 min.

Following incubation each sample was treated with 5 ml of 5% trichloroacetic acid and the precipitates were processed as described for RNA polymerase.

SDS-polyacrylamide gel electrophoresis of chromatin proteins—Chromatin samples containing 100 μg protein each were dissolved in 50 mM Tris-HCl, pH 6.8, 2% SDS, 10 mM 2-mercaptoethanol and 10% glycerol and boiled for at least 5 min. Then a tracking dye was added and the protein mixture was applied to a one-dimensional polyacrylamide gel, consisting of a 3% stacking gel, pH 6.8 and a 10% separating gel, pH 8.8. Both gels contained 0.2% recrystallized SDS and 750 mM Tris-HCl according to Laemmli (1970) as modified by Elgin (1975). Electrophoresis was performed at 3.5 mA/gel, until the tracking dye reached the bottom of the tube. The gels were fixed in 12% trichloroacetic acid and stained in a solution containing 0.1% Coomassie Brilliant Blue R-250 and a mixture of methanol : acetic acid : water (1 : 1 : 8 v/v). Destaining was by diffusion in a mixture of methanol, acetic acid and water (1 : 2 : 17 v/v) under constant shaking.

After densitometric scanning and photography, the gels were cut into 0.5 mm slices with a Mickle gel slicer. Each slice was placed on a glass fiber filter (GF/A Whatman) and dried at 100°C for 30 min. The radioactivity in each slice was measured by liquid scintillation techniques.

The molecular weights of the chromatin proteins were determined by comparison with diethylpyrocarbonate-induced polymers of bovine pancreatic RNase A (Kahl et al. 1979).

Results

Activation of different chromatin-bound protein phosphokinases in wounded potato tuber tissue—Wounding of quiescent white potato tuber tissue enhances the activity of chromatin-bound protein phosphokinases (Fig. 1). Two phases of the enhancement of wound-induced phosphokinase activity were observed. A doubling of the activity of resting tubers during the first 18 hr after injury is characteristic for the first phase. During the second phase the activity toward endogenous

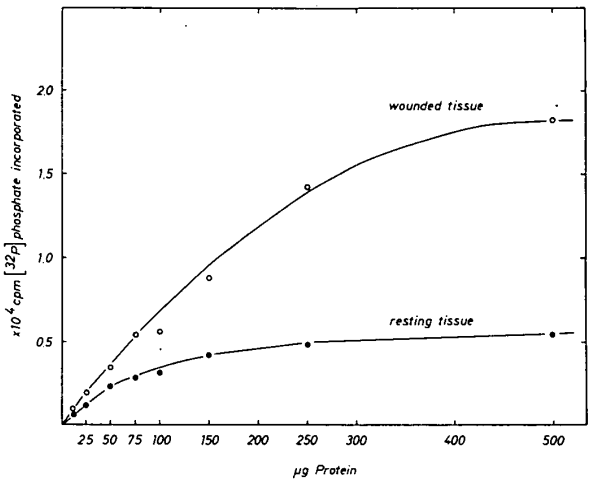

Fig. 1 Chromatin-bound protein phosphokinase activity from quiescent and 18 hr-wounded white potato tuber tissues as a function of chromatin protein concentration.

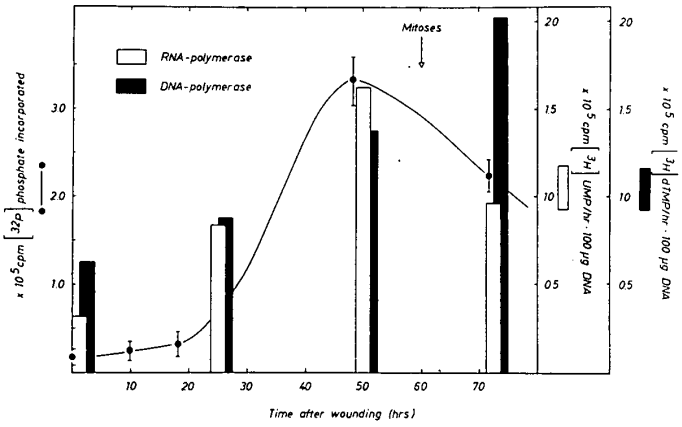

Fig. 2

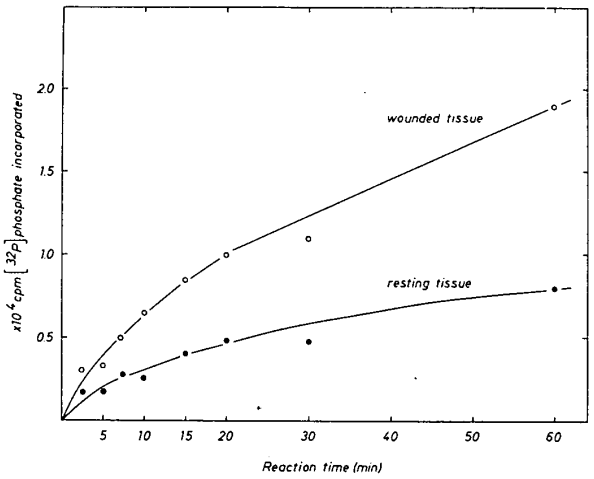

Fig. 3

Fig. 2 Wound-induced changes in chromatin-bound protein phosphokinase (-●-), DNA-dependent RNA polymerase (□) and DNA-dependent DNA polymerase (■) activities. The arrow indicates the onset of the first wave of mitoses.

Fig. 3 Time-course of protein phosphokinase activity in chromatin preparations from resting and 18 hr wounded potato tuber tissues. Phosphate acceptors were endogenous chromatin proteins.

proteins increases to a peak about 48 hr after injury, where it is 30 fold that of resting tissues, then declines. Even after 72 hr protein kinase activity is about 15-fold higher than that in quiescent tissues (Fig. 2). The term "endogenous proteins" comprises both basic proteins (mostly histones) and heterogeneous acidic proteins. The latter are referred to as nonhistone proteins.

If the chromatin-bound phosphokinases of resting tissue were supplied with histones from calf thymus nuclei, the phosphorylation rate was much lower than that of endogenous proteins and was only slightly enhanced after wounding. Similarly low phosphorylation was observed in experiments with phosvitin as exogenous substrate for the chromatin-bound enzymes. Again, the kinases of chromatin from wounded tissues were more active than those from unwounded controls. Phosphorylation of added casein was comparable to that of endogenous proteins (Fig. 3). Wounding roughly doubles the activity of the enzymes toward casein.

Characteristics of chromatin-bound protein phosphokinases of resting and wounded potato tuber tissue— Like phosphokinases from other sources, the chromatin-bound enzymes of wounded tissues were stimulated by Mg^{2+}. However, in the present experiments considerable activity could be detected without added Mg^{2+}. This may be brought about by residual Mg^{2+} firmly attached to the isolated and purified chromatin (Fig. 4). The requirements for Mg^{2+} of kinases from resting and wounded tissues were distinctly different. The former generally were not or only slightly stimulated by Mg^{2+}. This is true for the enzymes to which endogenous proteins as well as phosvitin or casein were added as substrates. The histone kinases, however, were stimulated about twofold at 100 mM Mg^{2+}, but neither exhibit any distinct optimum (Fig. 4B). On the other hand, the phosphokinases of chromatin from wounded tissues were optimally stimulated by 5 mM (casein kinases, Fig. 4D), 10 mM (endogenous protein kinases and phosvitin kinases Fig. 4A, C) and 50 mM $MgCl_2$ (histone kinases Fig. 4B). Thus, the Mg^{2+} requirement of the chromatin protein kinases changes strikingly after wounding (Fig. 4).

Ca^{2+} in the 1–20 mM range did not influence the phosphorylation of chromatin proteins from either quiescent or wounded tissues. Neither did spermine, spermidine (each 1–20 mM) nor 3′,5′-cyclic AMP (10^{-4}–10^{-8} M) show any positive effect on the activity of phosphokinases (data not shown).

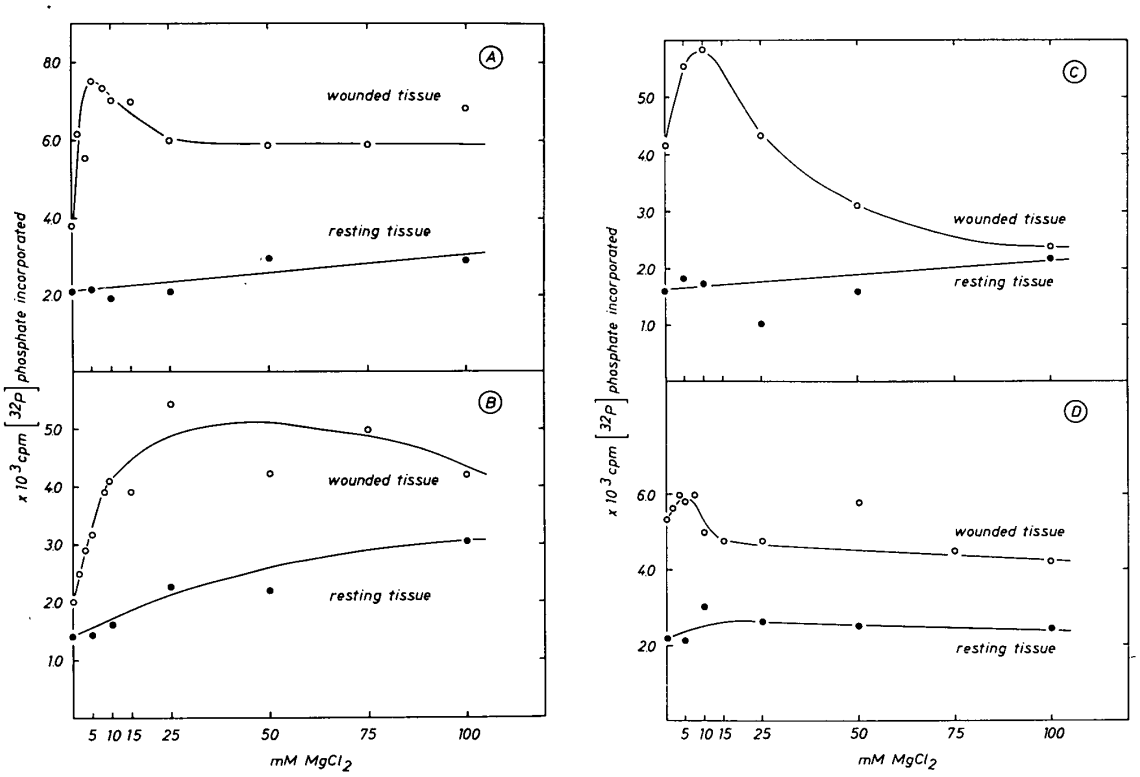

Fig. 4 Mg^{2+}-requirements of chromatin-bound protein phosphokinases from resting (-●-) and 18 hr wounded (-○-) potato tuber tissues. A, Endogenous proteins; B, calf thymus histones; C, phosvitin; D, casein as phosphate acceptors.

The phosphate acceptor in the kinase reaction is a protein. If samples of in vitro labeled chromatin were digested by RNase A or DNase I for 3 and 15 hr respectively, no significant amount of label becomes soluble. This proved that no or only negligible amounts of [γ-^{32}P]-ATP are incorporated into nucleic acids, i.e., during RNA chain initiation. However, trypsin in a 15 hr digestion period rendered most of the label soluble, indicating proteins as phosphate acceptors. The phosphate most probably is introduced into seryl- and/or threonyl residues of the acceptor proteins, because short incubation of these proteins in hot NaOH solubilized nearly all incorporated phosphate. Acyl phosphates evidently are not formed (hydroxylamine treatment), nor is phosphohistidine involved (succinic acid treatment; Table 1).

All phosphokinases were sensitive to *p*-hydroxymercuribenzoate, an effective inhibitor of SH-groups. Fifty percent inhibition of kinase activity was obtained with 2 mM *p*-hydroxymercuribenzoate in resting tissues and with only 0.3 mM in wounded tissues (Fig. 5). Thus phosphokinases from chromatin of resting tissues are definitely less sensitive.

Electrophoretic analysis of the phosphorylated proteins in resting and wounded tissues—If the proteins were dissociated from purified chromatin of resting tissues and electrophoresed under denaturing conditions on 10% polyacrylamide gels, about 40 protein bands with molecular weights ranging from less than 10,000 to over 100,000 daltons were separated. There was only weak and generally random incorporation of [γ-^{32}P] from ATP into these proteins, but with a tendency toward higher phosphorylation in the low molecular weight region (see dotted line in Fig. 6; note the 12,000 dalton component). The pattern of proteins was altered as a consequence of wounding. For example, the intensity of some of the bands in the low molecular weight region was changed. In the high molecular weight region, the pronounced heterogeneity of proteins from resting tissues is lost and three compounds with molecular weights of about 29,000, 72,000 and more than 85,000

Table 1 Sensitivity of $^{32}P_i$ in chromatin proteins from wounded potato tuber tissue toward enzymes and chemical agents

Reagent (conc)	Buffer	Length of treatment	Temperature (°C)	Residual radioactivity (%)
RNase A (50 μg/ml)	50 mM Tris-HCl pH 7.5	4 hr	37	90
DNase I (40 μg/ml)	50 mM Tris-HCl pH 7.5 containing 10 mM $MgCl_2$	15 hr	37	94
Trypsin (100 μg/ml)	50 mM Tris-HCl pH 7.5	24 hr	37	13
NAOH (1 N)	50 mM Tris-HCl pH 7.5	20 min	100	3
Hydroxylamine (0.8 M)	50 mM sodium acetate pH 5.4	10 min	20	96
Succinic acid (1.0 M)	50 mM sodium acetate pH 5.4	30 min	37	90

Chromatin samples (250 μg protein) were incubated in a scaled-up reaction mixture together with [γ-^{32}P]-ATP, which either contained 10 mM Tris-buffer, pH 7.5 or 50 mM sodium acetate buffer, pH 5.4. After 30 min the phosphorylation reaction in both mixtures was stopped at 100°C for 5 min and the chromatin solution divided and treated with enzymes or reagents as outlined in **Materials and Methods**. Each treatment was stopped by precipitation with 5 ml of 5% trichloroacetic acid. The radioactivity remaining on the filters after washing the samples extensively with 2% trichloroacetic acid is given as a percentage of that of the respective control.

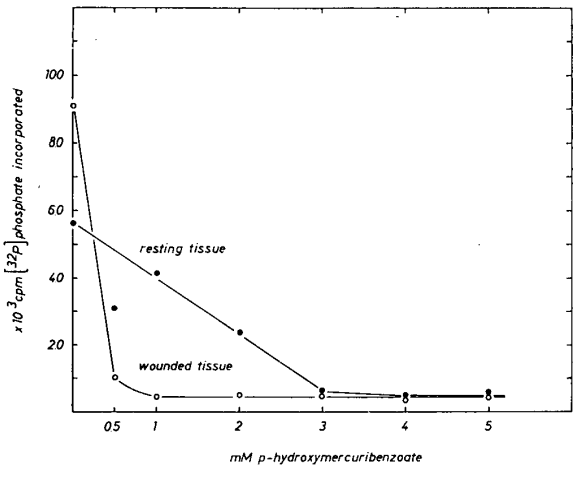

Fig. 5

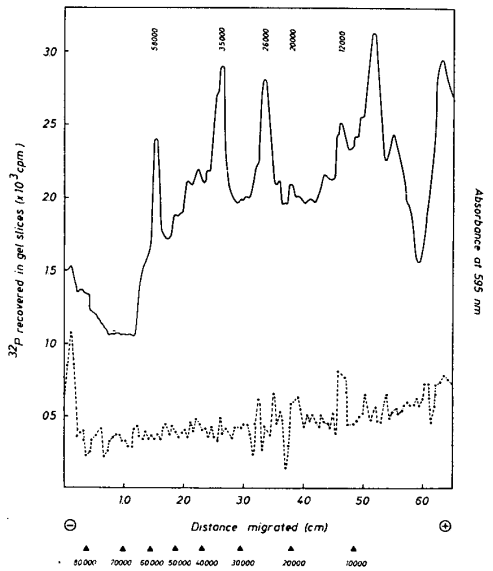

Fig. 6

Fig. 5 Dependence of chromatin-bound protein phosphokinase activity on SH-groups as titrated with increasing amounts of *p*-hydroxymercuribenzoate. Substrates were endogenous proteins.

Fig. 6 Electrophoretic analysis of the proteins phosphorylated in vitro by chromatin-bound protein phosphokinases from resting potato tuber tissue. The upper curve represents the densitometric scan of a Coomassie Brilliant Blue stained gel. The gel was cut into 0.5 mm slices. The radioactivity of each slice was measured by liquid scintillation techniques (dotted line). Arrow-heads indicate positions of molecular weight markers (see **Materials and Methods**).

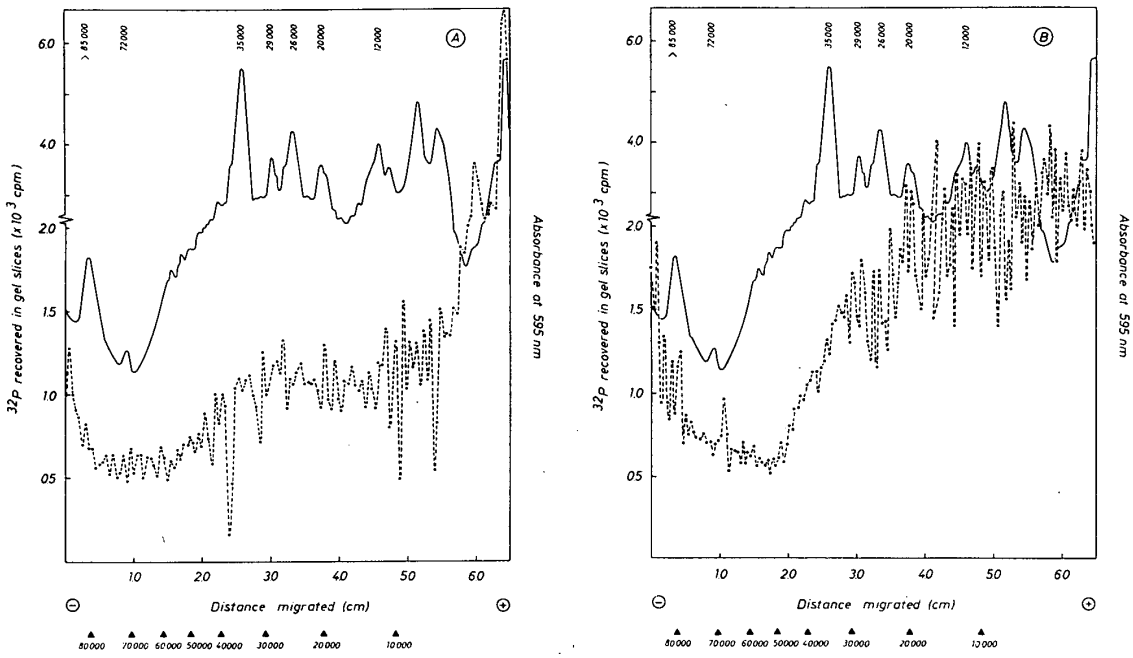

Fig. 7 Electrophoretic analysis of the proteins phosphorylated in vitro by chromatin-bound phosphokinases from wound-healing potato tuber tissue. A, 18 hr after wounding; B, 48 hr after wounding.

daltons became predominant. Moreover, the 58,000 dalton protein of chromatin from quiescent tissues vanished after wounding (Fig. 7A). These wound-induced changes in protein patterns are accompanied by an enhancement of protein phosphorylation, especially pronounced in the low molecular weight region. Even after 48 hr of wound-healing this pattern hardly changes. As expected, however, total phosphorylation increased with time after wounding (Fig. 7B). Thus, phosphorylation of chromosomal proteins is changed both qualitatively and quantitatively after wounding.

Discussion

The present experiments were designed to find out what qualitative changes occur in the nonhistone chromosomal proteins after wounding of white potato tuber tissue. These proteins, if extracted from resting tissues, do not reverse histone-inhibited polynucleotide synthesis catalyzed by *E. coli* RNA polymerase on a modified chromatin template. After wounding the nonhistone chromosomal proteins are able to counteract the histones in the same in vitro transcription system (Kahl et al. 1979). These changes in quality are possibly brought about by phosphorylation of the nonhistone chromosomal proteins. In fact, white potato tuber chromatin possesses protein phosphokinases even in intact tubers, where the cells are virtually quiescent (Fig. 1). Wounding leads to an increase in phosphokinase activity with a plateau after about 15–20 hr and a peak after 48 hr. This time-course of activity closely resembles that of chromatin-bound DNA-dependent RNA polymerase activity. These enzymes (both polymerases I and II being present and simultaneously activated) are stimulated about 5–6 fold after wounding with a peak at 48 hr. On the other hand, there is a concomitant and steady increase in chromatin-bound DNA-dependent DNA polymerase activity (both polymerases α and β being present; data not shown). A certain level of DNA polymerase activity has apparently to be reached in the potato system before mitoses are possible (Fig. 2; Watanabe and Imaseki 1977). It is unknown whether the phosphorylation of chromosomal proteins has anything to do with the onset of DNA synthesis.

No matter what substrates serve as phosphate acceptors (i.e. endogenous proteins, histones, casein or phosvitin), the phosphokinases from injured tissue always are more active than those from resting tissue. The present results do not allow a definite conclusion as to the activation mechanism, but they are interpreted as reflecting changes in the properties of the enzymes after wounding. The inability of Mg^{2+} even at very high concentrations to stimulate phosphokinase activity on chromatin from resting tissues and the pronounced effect of low $MgCl_2$ concentrations on the enzymes from chromatin of wounded tissues support this hypothesis (Fig. 4). This is true for all phosphokinase preparations, whatever substrate is used. Moreover, sensitivity toward agents which block SH-groups has changed after wounding. The enzymes from intact tissues are less susceptible to *p*-hydroxymercuribenzoate, but enzymes from wounded tissues are extremely sensitive. Taken together these results may mean that a new set of enzymes appears after wounding. In most tissues so far investigated at least two types of phosphokinases have been located on the chromatin (Trewavas 1975), which possibly have different nuclear function, i.e., protein substrate specificities. Changes in the relative concentrations of both enzymes are to be expected as a consequence of induction processes (i.e., after hormone-treatment: Murray et al. 1978a, b, c) and may be responsible for the wound-induced alterations in the properties of phosphokinases.

The quantity of chromosomal proteins also changes after wounding and more pronouncedly after hormone application (Kahl et al. 1979, Rosenstock and Kahl 1978). There is greater heterogeneity of chromosomal proteins in wounded tissues, especially in the high-molecular weight region. Phosphorylation of the chromosomal proteins is relatively low and uniform in resting tissues (Fig. 6). Wounding results in higher phosphorylative capacity of the chromatin-bound enzymes and an enhanced incorporation of ^{32}P from [γ-^{32}P]-ATP into chromosomal proteins, especially the low molecular weight fraction (Fig. 7). The pattern of phosphorylation of chromosomal proteins, then, is quantitatively and qualitatively different in the two types of tissues.

Chromosomal protein phosphorylation frequently precedes the induction of RNA transcription in normal and abnormal developmental processes as shown for slime molds, sea urchin embryos, lymphocytes, erythrocytes, fibroblasts, neurones and glial cells (Kleinsmith 1975b). Similar correlations seem to exist in soybean hypocotyls treated with 2,4-D (Murray and Key 1978a) and in germinating barley (Chapman et al. 1975, Van Loon et al. 1975). Nuclear protein phosphorylation alters the availability of specific templates for transcription (Kamiyama and Dastague 1974, Kleinsmith 1975a, Stein et al. 1975, Trewavas 1975). Moreover, protein kinases stimulate in vitro transcription of chromatin by RNA polymerases I and II (Dahmus 1976, Trewavas 1975). RNA polymerases generally seem to belong to the natural substrates of phosphokinases in yeast, ascites tumor cells and calf thymus (Dahmus 1976), although only in a few cases a stimulation of activity follows phosphorylation (ascites tumor RNA polymerase II: Dahmus 1976, calf thymus RNA polymerase II: Trewavas 1975). Although suggestive, the present data do not prove any such relationship between phosphorylation of distinct chromosomal proteins and the onset of transcription after wounding. The dramatic wound-induced increase of chromatin-bound phosphokinase activity with concomitant change in the properties of the kinases and the qualitative change in the phosphorylation pattern of chromosomal proteins parallels the enhancement of DNA-dependent RNA polymerase activity. This relationship, however, may be coincidental rather than causative. Thus, a definite conclusion as to the function of phosphorylated proteins in RNA synthesis of wounded plant tissues awaits further experiments.

This work was supported by Deutsche Forschungsgemeinschaft (grant Ka 332/7). W. Schäfer acknowledges a fellowship from the Fazit-Foundation (Frankfurt, Germany). We thank Mrs. S. Kost and Mrs. H. Schäfer for help during preparation of this manuscript.

References

Chapman, K. S. R., A. Trewavas and L. C. van Loon (1975) Regulation of the phosphorylation of chromatin-associated proteins in *Lemna* and *Hordeum*. *Plant Physiol.* 55: 293–296.

Dahmus, M. E. (1976) Stimulation of ascites tumor RNA polymerase II by protein kinases. *Biochemistry* 15: 1821–1829.

Elgin, S. C. R. (1975) Methods for the isolation and characterization of nonhistone chromosomal proteins. *Methods in Enzymol.* 40: 144–160.

Kahl, G. (1971) Synthesis of rRNA, tRNA and other RNA-species concomitant with polyribosome formation in aging potato tuber slices. *Z. Naturforsch.* 26b: 1058–1064.

Kahl, G. (1973) Genetic and metabolic regulation in differentiating plant storage tissue cells. *Bot. Rev.* 39: 274–299.

Kahl, G. and B. Wielgat (1976) Regulation of transcriptional activity in wounded potato tuber tissues. *Physiol. Vég.* 14: 725–738.

Kahl, G. and M. Wechselberger (1977) Activation of chromatin-bound DNA-dependent RNA polymerase (EC 2.7.7.6) in plant storage tissue slices. *Z. Naturforsch.* 32c: 229–235.

Kahl, G., W. Schäfer and M. Wechselberger (1979) Changes in nonhistone chromosomal proteins during the development of potato tubers; their involvement in wound- and hormone-induced processes. *Plant & Cell Physiol.* 20: 1217–1228.

Kamiyama, M. and B. Dastague (1974) Rat liver non-histone proteins: correlation between protein kinase activity and activation of RNA synthesis. *Biochem. Biophys. Res. Commun.* 44: 29–36.

Kleinsmith, L. J. (1973) Specific binding of phosphorylated nonhistone chromatin proteins to desoxyribonucleic acid. *J. Biol. Chem.* 248: 5648–5653.

Kleinsmith, L. J. (1975a) Phosphorylation of non-histone proteins in the regulation of chromosome structure and function. *J. Cell Physiol.* 85: 459–475.

Kleinsmith, L. J. (1975b) Do phosphorylated proteins regulate gene activity? *In* Chromosomal Proteins and Their Role in the Regulation of Gene Expression. Edited by G. S. Stein and L. J. Kleinsmith. p. 45–57. Academic Press, New York.

Kleinsmith, L. J., J. Stein and G. Stein (1976) Dephosphorylation of non-histone proteins specifically alters the pattern of gene transcription in reconstituted chromatin. *Proc. Natl. Acad. Sci. USA* 73: 1174–1178.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680–685.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951) Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275.

Murray, M. G. and J. L. Key (1978a) 2,4-Dichlorophenoxyacetic acid-enhanced phosphorylation of soybean nuclear proteins. *Plant Physiol.* 61: 190–198.

Murray, M. G., T. J. Guilfoyle and J. L. Key (1978b) Isolation and preliminary characterization of a casein kinase from cauliflower nuclei. *Plant Physiol.* 62: 434–437.

Murray, M. G., T. J. Guilfoyle and J. L. Key (1978c) Isolation and characterization of a chromatin-associated protein kinase from soybean. *Plant Physiol.* 61: 1023–1030.

Patel, G. L. and T. L. Thomas (1973) Some binding parameters of chromatin acidic proteins with high affinity of desoxyribonucleic acid. *Proc. Natl. Acad. Sci. USA* 70: 2524–2528.

Rosenstock, G. and G. Kahl (1978) Phytohormones and the regulation of cellular processes in aging storage tissues. *In* Biochemistry of Wounded Plant Tissues. Edited by G. Kahl. p. 623–671. Walter de Gruyter, Berlin.

Rubin, C. S. and O. M. Rosen (1975) Protein phosphorylation. *Ann. Rev. Biochem.* 44: 831–887.

Shea, M. and L. J. Kleinsmith (1973) Template specific stimulation of RNA synthesis by phosphorylated non-histone proteins. *Biochem. Biophys. Res. Commun.* 50: 473–477.

Splesberg, T. C., J. A. Wilhelm and L. S. Hnilica (1972) Nuclear proteins in genetic restriction II. The non-histone proteins in chromatin. *Subcell. Biochem.* 1: 107–145.

Stein, G. S., J. S. Stein and L. S. Kleinsmith (1975) Direct evidence for a functional relationship between non-histone chromosomal protein phosphorylation and gene transcription. *In* Chromosomal Proteins and Their Role in the Regulation of Gene Expression. Edited by G. S. Stein and L. J. Kleinsmith. p. 59–66. Academic Press, New York.

Trewavas, A. J. (1975) Post-translational modification of proteins by phosphorylation. *Ann. Rev. Plant Physiol.* 27: 349–374.

Van Loon, L. C., A. Trewavas and K. S. R. Chapman (1975) Phosphorylation of chromatin-associated proteins in *Lemna* and *Hordeum*. *Plant Physiol.* 55: 288–292.

Watanabe, A. and H. Imaseki (1977) Enhancement of DNA polymerase activity in potato tuber slices. *Plant & Cell Physiol.* 18: 849–858.

Wielgat, B. and G. Kahl (1979) Gibberellic acid activates chromatin-bound DNA-dependent RNA polymerase in wounded potato tuber tissue. *Plant Physiol.* 64: 867–871.

(Received August 10, 1981; Accepted November 18, 1981)