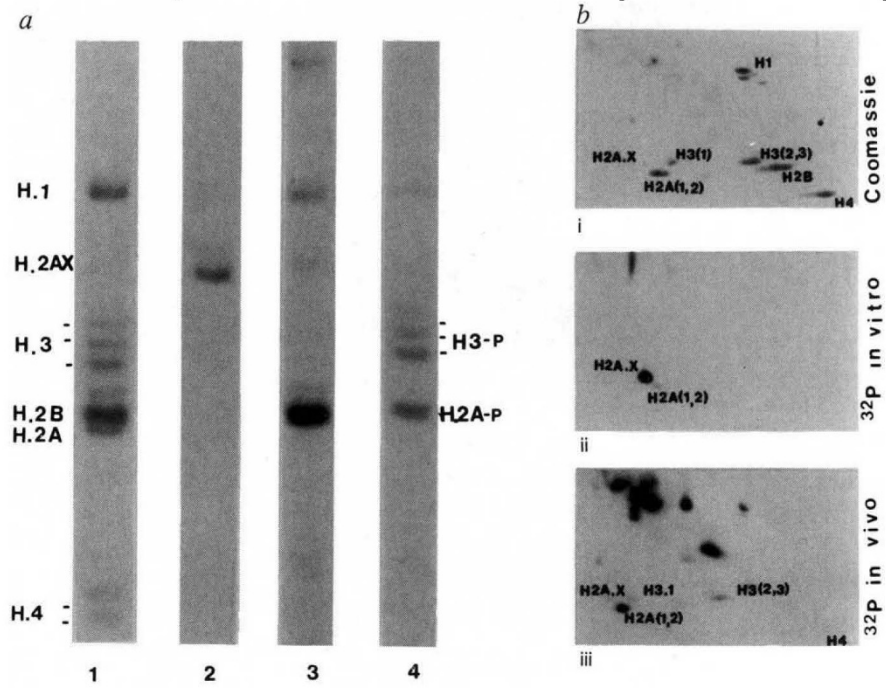
been replaced since 1990 by other commercially available artificial baits containing new generations of vaccines: the previously described SAG1 strain developed by the CNRS and Virbac, and the recombinant vaccinia virus containing the rabies glycoprotein gene (Rhône–Mérieux)[1]. Vaccination is extended to the whole contaminated area (112,000 km^2), half with each virus (*b* in the figure). In Switzerland, SAG1 vaccine has been used since spring 1991, whereas recombinant vaccinia has been chosen in Belgium. Other European countries still use classical fixed strains of rabies.

A. Flamand
P. Coulon
F. Lafay
Laboratoire de Génétique des Virus, CNRS, 91198 Gif sur Yvette Cedex, France
A. Kappeler
Swiss Rabies Centre, Institute of Veterinary Virology, University of Bern, 3012 Bern, Switzerland
M. Artois
M. Aubert
Laboratoire d'Etudes sur la Rage, CNEVA, BP9, 54220 Malzeville, France
J. Blancou
Office International des Epizooties, 12, rue de Prony, 75017 Paris, France
A. I. Wandeler
Agriculture Canada, Animal Diseases Research Institute, PO Box 11300, Station H, Nepean, Ontario K2H 8P9, Canada

Which histone kinase?

SIR — Growth factors, phorbol esters, okadaic acid and protein-synthesis inhibitors stimulate rapid phosphorylation of the nucleosomal core histone H3 (ref. 1), the duration and strength of which correlates well with induction and superinduction of the oncogenes c-*fos* and c-*jun*. To identify possible kinase(s) involved in this response we have repeated the experiments of Whitlock *et al.*[2], who described the existence of a chromatin-associated, calcium-dependent histone H3 kinase whose activity is enhanced in butyrate-treated cells. Although the phosphorylation phenomenon they described is entirely reproducible, the phosphoprotein involved is not histone H3, but a minor, then undiscovered, variant of H2A called H2A.X.

We prepared nuclei from quiescent and stimulated C3H 10T1/2 cells, and performed *in vitro* kinase assays using conditions identical to those described in ref. 2. Irrespective of whether cells were stimulated or not, the main ^{32}P-labelled phosphoprotein migrates just above the maximally acetylated forms of histone H3 on acid–urea gels (*a* in the figure and Fig. 2 of ref. 2). In agreement with Whitlock *et al.*[2], we find that this phosphoprotein comigrates exactly with histone H3 in SDS/PAGE gels and its phosphorylation is augmented in the presence of calcium and in nuclei derived from cells pretreated with sodium butyrate[2,3]. But this phosphoprotein did not comigrate on acid–urea gels (*a* in the figure) with phosphorylated histone H3 isolated from mitogen-stimulated cells labelled *in vivo* with ^{32}P-phosphate[1].



a, Acid–urea gels comparing *in vitro* and *in vivo* ^{32}P-labelled histones. Lane 1, Coomassie staining; lanes 2–4, autoradiograms of *in vitro*-labelled histones (lane 2), histones labelled *in vivo* from unstimulated cells (lane 3) and cells stimulated with 50 ng ml^{-1} epidermal growth factor, plus 10 μg ml^{-1} anisomycin for 1 h (lane 4). *b*, TAU/SDS two-dimensional electrophoresis[4] of the above. i, Coomassie blue staining; ii, ^{32}P-ATP *in vitro*-labelled histones, iii, ^{32}P-phosphate *in vivo*-labelled histones from stimulated cells.
METHODS. Cells were washed twice with ice-cold PBS and nuclei prepared either by lysis in 10 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol and 1% NP-40 (ref. 7), or exactly as described in ref. 2 with similar results. Nuclei were washed, ^{32}P-ATP-labelled and histones prepared as described[2]. ^{32}P-labelling and cell stimulation *in vivo* is described in ref. 1.

To resolve this problem we used a two-dimensional gel system, with Triton–acid–urea (TAU) gels[4] in the first dimension and SDS gels in the second, capable of resolving variant forms of the core histones (*b* in the figure). In particular, the inclusion of 0.37% Triton X-100 in one-dimensional gels allows unambiguous resolution of the main variants of histones H3 and H2A. This shows that the main *in vitro* phosphorylated species observed here and in ref. 2 is not histone H3 but H2A.X (ref. 5). Its mis-identification arises from its precise comigration with histone H3 in SDS gels and its migration close to hyperacetylated H3 in acid–urea gels.

It is intriguing that the *in vitro* kinase activity described here and in ref. 2 produces marked phosphorylation of the minor H2A.X variant, but negligible phosphorylation of H2A.1 and H2A.2 despite the large stoichiometric excess of these latter species[5]. Although most of the work on H2A.X phosphorylation has been done using *in vitro* models, DNA-dependent phosphorylation of H2A.X has been implicated in modulating nucleosome spacing in Xenopus oocytes[6]. We thank Ron Laskey (Wellcome/CRC Institute, Cambridge) for suggesting the possible identity of this phosphoprotein.

Eva Cano
Michael J. Barratt
Louis C. Mahadevan
Nuclear Signalling Laboratory, Developmental Biology Research Centre, King's College London, 26–29 Drury Lane, London WC2B 5RL, UK

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