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E. M. De Robertis, G. A. Partington and J. B. Gurdon

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Selective gene expression by somatic nuclei injected into amphibian oocytes

By E. M. De Robertis, G. A. Partington and J. B. Gurdon, F.R.S.
M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

The identification of the chromosomal molecules that regulate gene activity in eukaryotes has been hampered by the lack of experimental systems in which their biological activity can be assayed. We summarize here recent work from our laboratory which indicates that *somatic nuclei* introduced into amphibian oocytes express their genes in a highly selective and biologically meaningful way. The experimental system described provides one way in which the activity of genes and associated molecules can be assayed.

Somatic nuclei are prepared for injection by gentle procedures (Gurdon 1976), and a suspension of about 200 nuclei is introduced into each oocyte. During the first few days in oocytes, the somatic nuclei enlarge enormously (from 10- to 100-fold; see Gurdon, De Robertis & Partington 1976*a*). During this period the nuclei exchange their proteins with those of the surrounding cytoplasm, taking up large amounts of oocyte histone and non-histone proteins (Gurdon, Partington & De Robertis 1976*b*). The nuclei are very active in RNA synthesis, do not divide, and can be kept in culture for extended periods of time (Gurdon *et al.* 1976*a*, *b*).

Morphologically, the injected nuclei tend to resemble the oocyte's nucleus (germinal vesicle). For example, they stain with light green and contain dispersed chromatin (which sometimes forms threads reminiscent of chromosome-like structures). These similarities induced us to explore the possibility that this resemblance might extend beyond the morphological level, so that the choice of individual genes which are active in injected nuclei may come to be the same as those which are active in oocyte nuclei.

Fortunately very sensitive methods for the identification of proteins have recently become available (O'Farrell 1975). This has allowed us to analyse the messenger activity of the RNAs transcribed by the somatic nuclei inside the oocytes. An example of this type of analysis is shown in figure 1*a* in which a protein that is induced by the injection of HeLa nuclei is arrowed (discussed in detail by De Robertis, Partington, Longthorne & Gurdon 1977). Figure 1*b* shows that this protein is not synthesized in the presence of α-amanitin. This inhibitor of eukaryotic RNA polymerase was used at an intracellular concentration of 10 μg/ml, previously found (Colman 1975) not to affect the synthesis of 28*S*, 18*S*, 5*S* and 4*S* RNA in *Xenopus* oocytes. As shown in figure 2, α-amanitin does not affect the translation of endogenous and injected mRNAs in oocytes.

By using these methods of protein analysis, we have recently been able to show that the oocyte cytoplasm is able to *reprogram* the injected nuclei, so as to conform to the oocyte pattern of gene expression. These experiments, described in detail by De Robertis & Gurdon (1977), involved the injection of *Xenopus laevis* somatic nuclei obtained from a cloned cell line of *Xenopus* kidney cells. These cells do not express several proteins that are normally present in *Xenopus* oocytes; but they do synthesize many proteins expressed in both types of cells, as well as other proteins that are present only in cultured cells but not in oocytes. The *Xenopus* cultured cell nuclei were injected into oocytes of a newt, *Pleurodeles waltlii*, which provides a different

protein background. The proteins induced by the *Xenopus* somatic nuclei were analysed by methods similar to those shown in figure 1. The results from this experiment (De Robertis & Gurdon 1977) showed that:

(*a*) Several *Xenopus* oocyte-specific proteins are expressed – that is, proteins normally synthesized by *Xenopus* oocytes but *not* by the cultured cells used as nuclear donors.

(*b*) Several proteins normally synthesized in both types of cells are also expressed.

(*c*) None of the cultured cell-specific proteins is detectable.

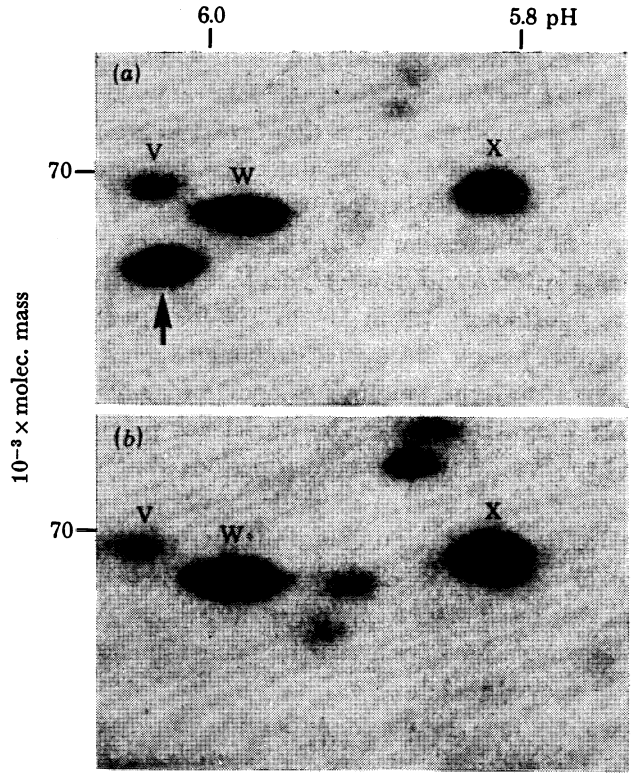

FIGURE 1. Radioactive proteins of *Xenopus* oocytes injected with HeLa nuclei and with or without α-amanitin. The oocytes were labelled for 6 h with [^{14}C]amino acids on the third day after injection. (*a*) Proteins of oocytes injected with HeLa nuclei. The arrow indicates a HeLa protein, which is absent in mock-injected oocytes. (*b*) Proteins from oocytes injected with HeLa nuclei and with α-amanitin at day 0, but in other respects treated in the same way for (*a*) above. Some oocyte proteins have been lettered V, W and X to help comparison.

We conclude from these experiments that amphibian oocytes contain components which are able to *reprogram* gene expression by the injected nuclei, in the absence of cell division; this involves a turning on of oocyte-genes that were previously inactive in the somatic nuclei. Gene expression by injected nuclei is therefore a highly selective and biologically meaningful process. Gene transcription and translation can also be obtained after injecting purified DNAs into oocytes, and this work is reviewed in this volume by Gurdon, Wyllie & De Robertis (1978). We believe that injected oocytes, which provide the natural conditions of living cells in an experimentally analysable situation, may be able to contribute to a better understanding of eukaryotic transcription.

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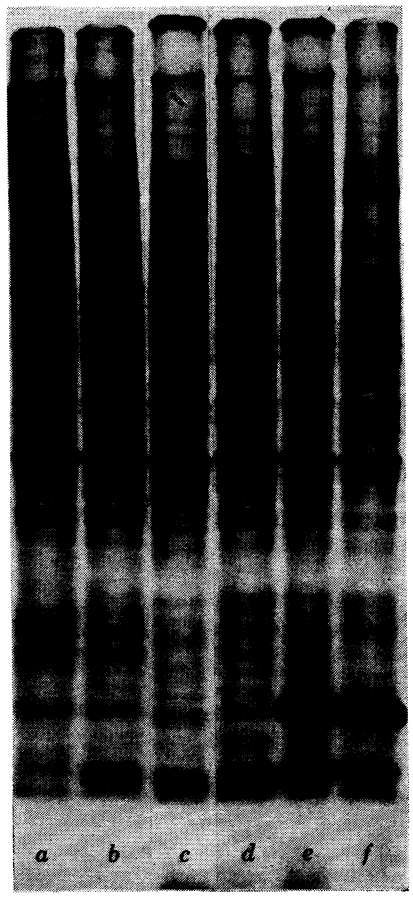

FIGURE 2. Lack of effect of α-amanitin on oocyte protein synthesis. Each oocyte was injected, as indicated below, with 200 HeLa nuclei, with α-amanitin to give an intracellular concentration of 10 μg/ml, or with 6 ng of rabbit globin mRNA; oocytes were then cultured for 3 days, labelled with a [^{14}C]amino acid mixture for 6 h, and their proteins electrophoresed on a one dimensional SDS slab gel and fluorographed.

type of injection	*a*	*b*	*c*	*d*	*e*	*f*
α-amanitin	−	+	−	+	−	+
HeLa nuclei	−	−	+	+	+	+
globin mRNA	−	−	−	−	+	+

It may be noted that the new proteins induced by the injection of HeLa nuclei are not detectable by standard one dimensional SDS electrophoresis. The samples in slots *c* and *d* are the same ones as shown in figure 1*a* and *b*. The arrow indicates the position of globin.

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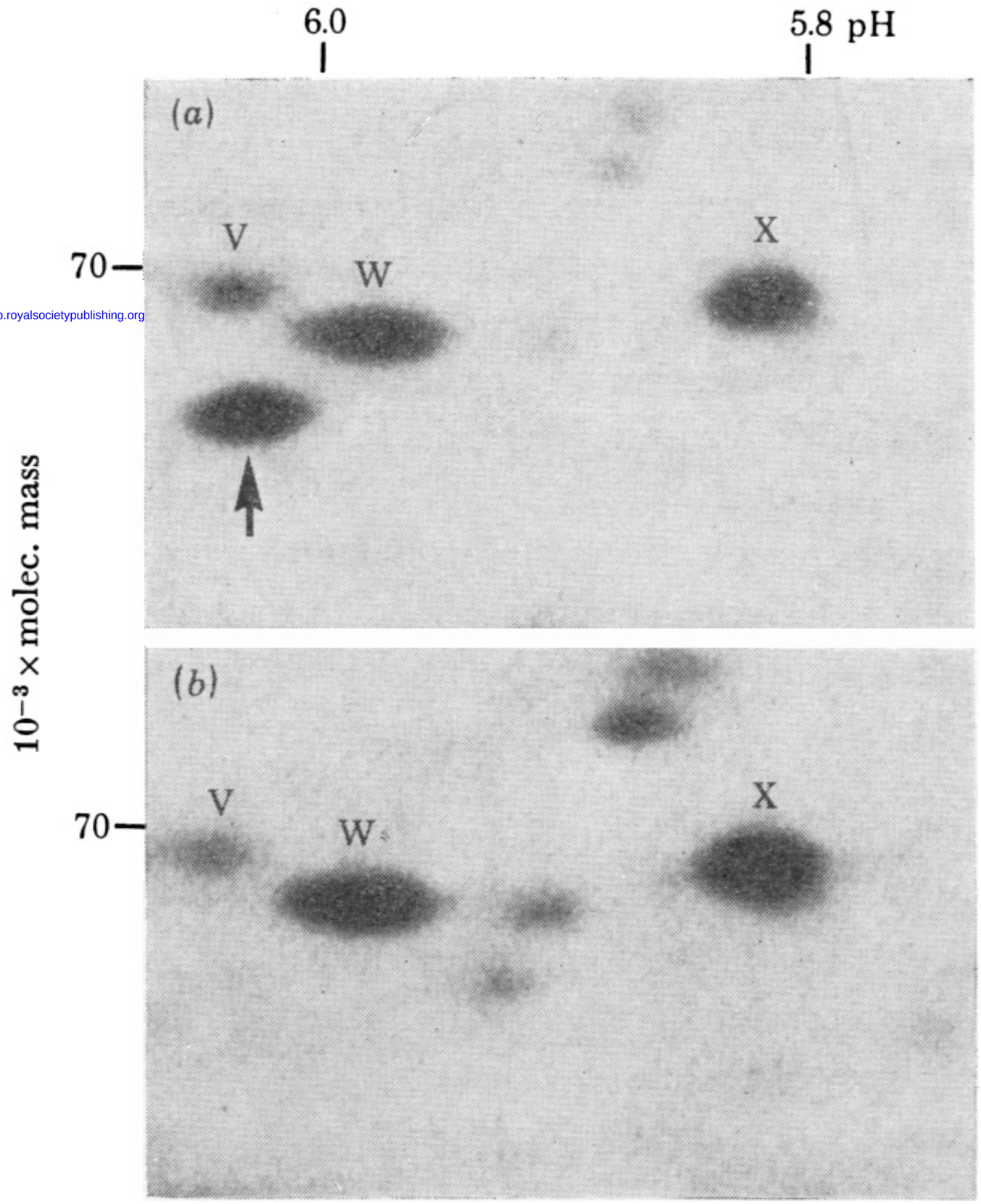
O'Farrell, P. 1975 *J. biol. Chem.* **250**, 4007–4021.



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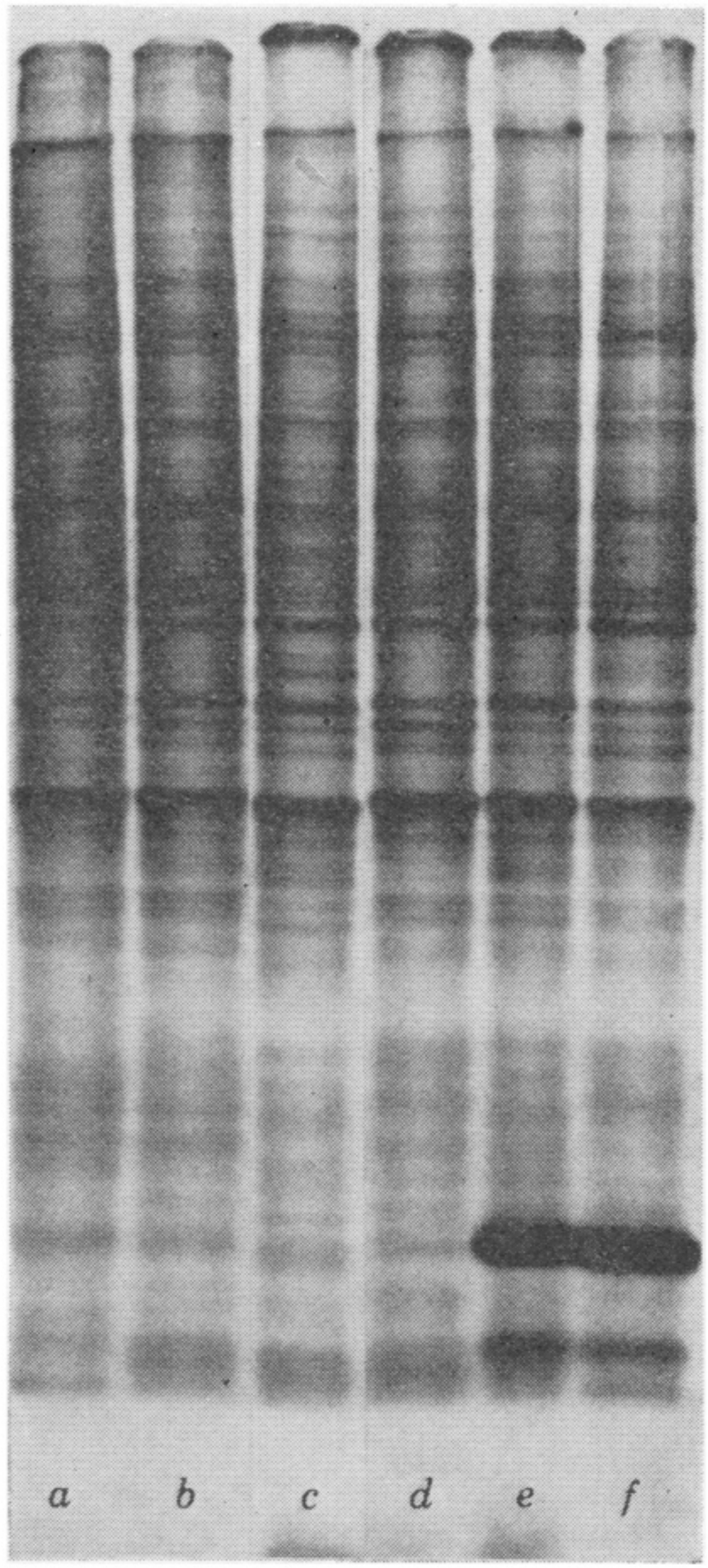

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