# Effects of gibberellic acid and abscisic acid on levels of translatable mRNA (1→3,1→4)-β-D-glucanase in barley aleurone

John Mundy and Geoffrey B. Fincher*

*Department of Biotechnology, Carlsberg Research Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark* and **Department of Biochemistry, La Trobe University, Bundoora, Victoria 3083, Australia*

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Polyadenylated mRNA has been isolated from barley aleurone layers incubated in the presence and absence of exogenous gibberellic acid and abscisic acid. Immunoprecipitation of in vitro translation products with specific antibodies shows that low levels of translatable mRNA encoding (1→3,1→4)-β-D-glucanase 4-glucanohydrolase (EC 3.2.1.73) can be detected in the absence of the phytohormones. Gibberellic acid causes a 10-fold increase in levels of translatable mRNA for the enzyme, while abscisic acid treatment suppresses the relative abundance of translatable (1→3,1→4)-β-D-glucanase mRNA.

*(Barley) Aleurone β-Glucanase translatable mRNA Gibberellic acid Abscisic acid*

## 1. INTRODUCTION

The major polysaccharide constituents of barley endosperm cell walls are the (1→3,1→4)-β-glucans [1]. During germination, these cell walls are degraded in a process which allows hydrolytic enzymes secreted from the surrounding aleurone and scutellar layers to penetrate more easily to the storage polymers inside cells of the starchy endosperm. Two (1→3,1→4)-β-glucan 4-glucanohydrolases (EC 3.2.1.73) purified from extracts of germinating barley exhibit a high degree of amino acid sequence homology (approx. 90%) and appear to be derived from separate genes which originated by duplication of a common ancestral gene [2]. Isoenzyme I, which has an $M_r$ of 28000, a p*I* of 8.5 and contains approx. 0.7% associated carbohydrate [3], is a major isoenzyme secreted from isolated scutella but is also detected in isolated aleurone layers [4]. Isoenzyme II has an $M_r$ of 30000, a p*I* higher than 10, contains approx. 4% carbohydrate [3] and is the predominant isoenzyme secreted from isolated aleurone layers [4]. Isoenzyme II secretion from aleurone layers is enhanced significantly by the phytohormone gibberellic acid [4]. Immunological studies suggest that a third (1→3,1→4)-β-glucanase may also be secreted from isolated scutella [4].

Thus, the synthesis of (1→3,1→4)-β-glucanase isoenzymes in germinating barley appears to be a relatively simple system for the study of tissue-specific regulation of gene expression and for the investigation of hormone action at a genetic level. Here, we assess the effects of gibberellic acid (GA) and abscisic acid (ABA) on levels of translatable mRNA for (1→3,1→4)-β-glucanase in barley aleurone by immunoprecipitation of in vitro translation products with specific antibodies.

## 2. MATERIALS AND METHODS

### 2.1. *Preparation and incubation of aleurone layers*

Aleurone layers were isolated from *Hordeum vulgare* L. cv. Nudinka essentially as described by Chrispeels and Varner [5]. 2000 embryo-less half-

* To whom correspondence should be addressed

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grains were surface sterilized in 0.2% (w/v) $AgNO_3$ [6] and imbibed for 4 days on moist, sterile filter paper. Starchy endosperm tissue was pressed out of the half-grains and the aleurone layers incubated for 20 h in 1 mM $CaCl_2$ with and without 10 $\mu$M $GA_3$ or 50 $\mu$M ABA.

### 2.2. *Isolation of mRNA*

The aleurone layers were ground under liquid nitrogen, thawed into 10 vols 10 mM Tris-HCl, pH 8.5 (containing 5 M guanidinium chloride, 5 mM EGTA, 100 mM $\beta$-mercaptoethanol, 0.1% lauryl sarcosine and 0.01% octanol) [7], and further homogenised in a Sorvall Omnimixer for 2.5 min at maximum speed. Total RNA was isolated from the extract by phenol treatment and ethanol precipitation. Poly(A)$^+$ mRNA was purified by affinity chromatography on poly(U)-Sepharose 4B (Pharmacia) [6].

### 2.3. *Immunoprecipitation of in vitro translation products*

Translations of mRNA in vitro were performed in the rabbit reticulocyte lysate system [8] as described in [6]. Lysate N.90 and L-[$^{35}$S]methionine (1300 Ci·mmol$^{-1}$) were from Amersham. Precursor polypeptides of (1→3,1→4)-$\beta$-glucanases were immunoprecipitated from total in vitro translation products according to Jonassen et al. [9] using polyclonal antibodies raised against (1→3,1→4)-$\beta$-glucanase isoenzyme I [3]. The antibodies cross-react with isoenzymes I, II and III [4].

Total in vitro translation products ($10^5$ cpm per lane) and polypeptides immunoprecipitated from $10^6$ cpm of total translation products were separated by electrophoresis in 12.5% polyacrylamide gels containing 1% SDS [10]. $M_r$ calibration markers were from Amersham (mixture CFA.626). Gels were treated with sodium salicylate [11] prior to fluorography.

## 3. RESULTS AND DISCUSSION

The yields of mRNA from untreated aleurone layers, ABA-treated aleurone layers, $GA_3$-treated layers and layers incubated with both ABA and $GA_3$ were 72, 84, 56 and 96 $\mu$g/1000 seeds, respectively. Although mRNA yields may be affected by the ease of extraction of mRNA from the treated aleurone layers or the extent of ribosomal RNA contamination of the preparations, these values parallel the levels of total transcription in nuclei isolated from hormone-treated barley aleurone protoplasts, where $GA_3$ suppresses total transcription and ABA counteracts this $GA_3$ effect [12].

The in vitro translation products of mRNAs extracted from hormone-treated barley aleurone layers are compared in fig.1A. A large number of polypeptides are synthesized when the translation system is programmed with mRNA from untreated layers, and striking differences are apparent with hormone treatment. In particular, a prominent

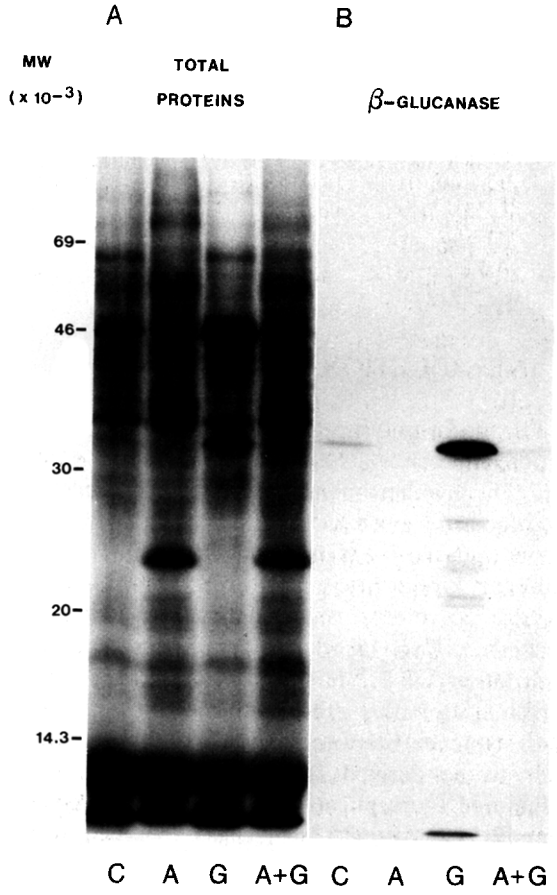


Fig.1. Fluorogram of SDS-polyacrylamide gel showing (A) total in vitro translation products of mRNA isolated from aleurone treated with phytohormones and (B) polypeptides immunoprecipitated from in vitro translation products with antibodies raised against (1→3,1→4)-$\beta$-glucanase. Lanes C, control (aleurone incubated without exogenous hormones); A, 50 $\mu$M ABA; G, 10 $\mu$M $GA_3$; A + G, both ABA and $GA_3$.

polypeptide of apparent $M_r$ 25000 is synthesized from mRNA of ABA-treated layers. Although the identity of this polypeptide has not yet been reported, it has been observed previously amongst the in vitro translation products of mRNA isolated from ABA-treated barley [12,14] and corresponds in terms of $M_r$ with major protein component in tissue extracts of ABA-treated barley aleurone [13,15].

Treatment of the aleurone layers with $GA_3$ increases levels of translatable RNA for a polypeptide of $M_r$ 46000 (fig.1A), which is presumably the $\alpha$-amylase precursor commonly observed amongst in vitro translation products of $GA_3$-treated barley aleurone [13,14,16]. Following treatment of the aleurone layers with both $GA_3$ and ABA, the abundance of this polypeptide amongst in vitro translation products decreases dramatically (fig.1; cf. [13,14,17]).

When the in vitro translation products from untreated aleurone layers were immunoprecipitated with antibodies to (1→3,1→4)-$\beta$-glucanase, a polypeptide of $M_r$ approx. 33000 was observed (fig.1B). This is consistent with the observation that relatively low levels of (1→3,1→4)-$\beta$-glucanase activity are secreted from isolated aleurone layers incubated without added phytohormones; most of the activity is due to isoenzyme II [4]. The polypeptide of $M_r$ 33000 could not be detected when in vitro translation products of mRNA from ABA-treated aleurone were immunoprecipitated with the antibody, but increased significantly in abundance in products of mRNA from $GA_3$-treated layers (fig.1B). Densitometric analysis of the autoradiogram (fig.1B) showed that the intensity of the immunoprecipitated polypeptide band of $M_r$ 33000 with $GA_3$ was approx. 10-fold greater than that of the control (no $GA_3$). Since $10^6$ cpm of total in vitro translation products were immunoprecipitated in each case, it is apparent that the relative abundance of translatable mRNA encoding (1→3,1→4)-$\beta$-glucanase increases significantly following $GA_3$ treatment of the aleurone layers. The minor, lower $M_r$ bands in fig.1B (lane G) are believed to result from contaminants in the antibody preparation [6]. When aleurone layers were incubated with both ABA and $GA_3$, levels of (1→3,1→4)-$\beta$-glucanase precursor polypeptide which could be immunoprecipitated from mRNA in vitro translation products decreased to those observed in untreated layers (fig.1B). Similar hormonal effects on a polypeptide of $M_r$ 33000 can be observed amongst the total in vitro translation products.

The effect of $GA_3$ in enhancing levels of translatable mRNA for (1→3,1→4)-$\beta$-glucanase is consistent with its influence at the enzyme level, where a 5–10-fold increase in (1→3,1→4)-$\beta$-glucanase II secretion from isolated aleurone layers of barley has been observed [4].

Thus, the hormonal regulation of levels of translatable mRNA encoding the (1→3,1→4)-$\beta$-glucanase of barley aleurone appears to parallel the regulation of translatable mRNA for $\alpha$-amylase; levels are increased by $GA_3$, but this effect is blocked by the simultaneous addition of ABA. In isolated aleurone layers, $GA_3$ may actually decrease the overall number of both proteins synthesized [13] and mRNA transcripts present [12], while re-directing gene expression and protein synthesis towards the rapid production of a small number of proteins. The evidence presented here suggests that, in addition to $\alpha$-amylase, (1→3,1→4)-$\beta$-glucanase is a member of the group of GA-stimulated proteins in barley aleurone.

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