

# A Comparison of the Effect of Salt on Polypeptides and Translatable mRNAs in Roots of a Salt-Tolerant and a Salt-Sensitive Cultivar of Barley

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## ABSTRACT

The effect of salt stress on polypeptide and mRNA levels in roots of two barley (*Hordeum vulgare* L.) cultivars differing in salt tolerance (cv CM 72, tolerant; cv Prato, sensitive) was analyzed using two-dimensional polyacrylamide gel electrophoresis. Preliminary experiments indicated that germination of Prato was inhibited significantly in the presence of NaCl, but growth of the surviving Prato seedlings was not substantially different from that of CM 72. Fluorographs of two-dimensional gels containing *in vivo* labeled polypeptides or *in vitro* translation products were computer analyzed to identify and quantitate changes that resulted when plants were grown in the presence of 200 millimolar NaCl for 6 days. The patterns of *in vivo* labeled polypeptides and *in vitro* products of CM 72 and Prato were qualitatively the same. Salt caused quantitative changes in numerous polypeptides and translatable mRNAs, but, overall, the changes were relatively small. Salt did not induce the synthesis of unique polypeptides or translatable mRNAs and did not cause any to disappear. Because of the similarities of the two cultivars with respect to growth and polypeptide patterns and the slight changes in polypeptide and translation product levels caused by salt, specific polypeptides or translatable mRNAs that are related to salt tolerance in barley could not be identified.

Salt tolerance of plants is a complex phenomenon that involves biochemical and physiological processes as well as morphological and developmental changes (4, 7). The underlying molecular/genetic mechanisms for salt tolerance have not yet been fully identified or characterized. One reason for this is that salt treatment elicits a number of responses in plants that range from readjustment of transport (5, 12) and metabolic processes (1) to inhibition of growth (13). One approach in studying the molecular mechanisms involved in this complex response is to use 2D<sup>1</sup> PAGE to identify polypeptides whose levels increase as a result of salt stress with the assumption that they have a role in salt tolerance. The identification of these polypeptides on 2D gels can be a first step in the isolation of genes that are regulated by salt. In previous studies, we have shown (9, 10) that salt stress caused increases or decreases in the synthesis of a number of polypeptides in roots of CM 72, a salt-tolerant cultivar of barley. The analysis

<sup>1</sup> Abbreviations: 2D, two-dimensional; pI, isoelectric point.

of two closely related genetic lines differing in salt tolerance provides an opportunity to determine if any of the polypeptides whose levels increase with salt are related genetically to salt tolerance. Barley cultivars vary widely in their ability to tolerate salt (2). For example, when emergence of the first leaf through the coleoptile sheath was examined as a function of NaCl concentration, Epstein *et al.* (3) found that California Mariout, the most tolerant cultivar in the analysis, had 100% emergence at 400 mM NaCl, whereas Arivat, the least tolerant cultivar, showed no emergence at this concentration. In this study, CM 72 and Prato were chosen for comparison because the original lines, California Mariout and Arivat, are no longer available. With respect to salt tolerance, CM 72 closely resembles California Mariout and Prato closely resembles Arivat.

In this paper, we report the effect of NaCl on germination and growth of CM 72 and Prato. We also report the effect of NaCl on polypeptides and translatable mRNAs in these two cultivars. Because a method for high resolution of plant proteins on 2D gels was used (8) to examine polypeptides and translation products, computer analysis could be used to detect polypeptides and translation products whose synthesis was modulated by salt treatment and to quantitate precisely these changes. Comparisons of CM 72 and Prato have been published previously (16-18), but changes attributed to salt treatment and developmental state were based on visual inspection of 2D gels that lacked high resolution. Accurate interpretations of data obtained by 2D PAGE can only be made when the gels have high resolution. The detailed comparisons reported in this paper were made possible by coupling high resolution 2D PAGE with the sensitivity of computer analysis.

## MATERIALS AND METHODS

### Plant Material

Seeds of barley (*Hordeum vulgare* L. cv CM 72 [N. F. Davis Drier & Elevator, Inc., Firebaugh, CA] or cv Prato [Foundation Seed, University of California, Davis, CA]) were sown above an aerated solution containing full strength nutrients (2) with or without 200 mM NaCl as described previously (9). Plants were grown at 100% humidity and 22°C in the dark for 6 d.

### Measurement of Percent Germination and Growth

For each treatment, approximately 100 seeds of CM 72 (5 g) or of Prato (4 g) were sown above nutrient solutions containing 0, 50, 100, 200, 300, or 400 mM NaCl. Percent germination, defined in this paper as emergence of roots through the seed coat, was determined for the total number of seeds sown per treatment. Lengths of shoots and roots (average length of all roots per plant) were determined to the nearest mm for 25 plants per treatment.

### In Vivo Labeling

Proteins were labeled *in vivo* with [<sup>35</sup>S]methionine as described previously (9), and the proteins were prepared for isoelectric focusing as described below. Roots of intact plants were labeled to avoid wound-induced changes in protein synthesis that could occur in excised root segments. In a preliminary experiment, we found that the 2D gel patterns of polypeptides labeled in roots of intact plants and in 1 cm root segments were different (WJ Hurkman, CK Tanaka, NL Robinson, unpublished observations).

### Preparation of Total RNA

To minimize degradation of the RNA during the isolation procedure, mortars and pestles, microfuge tubes, and all glassware were base-washed (0.1 N NaOH/20 mM EDTA), acid-washed (0.1 N HCl), rinsed thoroughly with deionized water, and autoclaved. In addition, all glassware was silanized (2.5% dichlorodimethylsilane in chloroform). All solutions and buffers were filtered (0.22  $\mu$ m filters) and autoclaved. However, the 10% SDS and 4 M LiCl stock solutions were filter-sterilized and heated to 80°C. Roots (10–15 g) were harvested, rinsed with sterile water, and blotted dry. The roots were ground to a fine powder in liquid nitrogen using a mortar and pestle. Extraction reagent (55 mL), made of equal volumes of PCI (phenol/chloroform/isoamyl alcohol, 49:49:2) and NTES (10 mM NaCl, 10 mM Tris-Cl [pH 8.5], 1 mM EDTA, 1% SDS), was added to the powder. A homogenate was obtained by continuous grinding as the mortar warmed to room temperature. When the homogenate reached approximately 4°C, it was transferred to capped Corex tubes (25 mL, Corning)<sup>2</sup> and the tubes were shaken vigorously for 5 min at 4°C. Following centrifugation at 12,000 *g* and 15°C for 15 min, the aqueous phase was removed and reextracted with an equal volume of PCI. Total nucleic acids were precipitated by addition of 0.1 volume of 2 M sodium acetate (pH 5.5) and 2.5 volumes of cold (–20°C) ethanol. Following overnight incubation at –20°C, precipitated nucleic acids were collected by centrifugation at 12,000 *g* and –2°C for 30 min. The precipitate was rinsed with cold 75% ethanol and dissolved in 7 mL of cold (4°C) sterile water. An equal volume of cold (4°C) 4 M lithium chloride (Chelex-100 treated, Bio-Rad) was added. Following incubation on ice for at least 3 h, the solution was centrifuged at 12,000 *g* and 4°C for 20 min. The supernatant, which contained the DNA, was discarded. The RNA pellet was

rinsed with cold (4°C) 2 M lithium chloride, and the pellet was suspended in 4.5 mL of cold sterile water. The RNA was precipitated out of the supernatant by addition of sodium acetate and ethanol as described above. Following overnight incubation at –20°C, the RNA was collected by centrifugation (12,000 *g* and 4°C for 10 min), and the pellet was rinsed with cold 75% ethanol. The RNA was suspended in 1 mL of cold sterile water. To remove any residual polysaccharides, the RNA was microfuged at 12,000 *g* and 4°C for 30 min. Typical yields were 3 to 6 mg of total RNA from 10 to 15 g of roots with  $A_{260}/A_{280}$  of 1.8 to 2.5 and  $A_{260}/A_{230}$  of 2.5. The integrity of the RNA was determined by the absorbance spectrum from 200 to 300 nm and by electrophoresis in 1.8% agarose gels containing formaldehyde.

### Isolation of Poly(A)<sup>+</sup> RNA

Poly(A)<sup>+</sup> RNA was isolated from total RNA by oligo(dT)-cellulose chromatography. The RNA solution, containing 2.5 to 5 mg of RNA, was heated at 65°C for 5 min and cooled on ice. The RNA solution was made equivalent to binding buffer (0.5 M LiOAc, 20 mM Tris-HCl [pH 7.5], 1 mM EDTA, 0.5% SDS) in a final volume of 1 mL. Half of the RNA solution was loaded onto a 1.2 mL oligo(dT)-cellulose (BRL) column that was equilibrated with binding buffer. The RNA solution was run into the column and incubated for 5 min; the remaining RNA solution was applied in the same manner. The column was then rinsed with 1 mL of binding buffer. The eluents from the RNA solutions and the binding buffer, which were collected in the same tube, were heated at 65°C for 3 min and run through the column again. This step was repeated once more, and the column was then rinsed with 8 mL of binding buffer. Bound poly(A)<sup>+</sup> RNA was eluted at 65°C with 6 mL of low salt buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA, 0.05% SDS) and collected in 1 mL fractions; fractions 2 through 5 contained the poly(A)<sup>+</sup> RNA. The poly(A)<sup>+</sup> RNA was precipitated as described above with sodium acetate and ethanol. Following incubation overnight at –20°C, the poly(A)<sup>+</sup> RNA was pelleted by centrifugation at 12,000 *g* and 4°C for 45 min. The pellet was suspended in cold sterile water (1  $\mu$ g RNA/ $\mu$ L water) and stored at –20°C.

### In Vitro Translation

Poly(A)<sup>+</sup> RNA was translated in an RNA-dependent, nuclease-treated, rabbit reticulocyte lysate system (Promega). The RNA was heated at 65°C for 3 min prior to translation and immediately placed on ice. Typically, 1  $\mu$ g of poly(A)<sup>+</sup> RNA was translated in a 25  $\mu$ L reaction volume that contained the reticulocyte lysate, amino acids less Met, and 25  $\mu$ Ci of [<sup>35</sup>S]methionine (approximately 1100 Ci/mmol or 41 TBq/mmol; New England Nuclear). The reaction was carried out at 30°C for 1 h. Incorporation of label into product was linear with poly(A)<sup>+</sup> RNA concentrations to 1.5  $\mu$ g and with time during the 1 h incubation period. The translation reactions were placed on ice and immediately processed for isoelectric focusing. When poly(A)<sup>+</sup> RNA was not added to the reaction, translation products were not detected by fluorography. In addition, when the reaction was carried out with poly(A)<sup>–</sup> RNA, no translation products were detected.

<sup>2</sup> Mention of a specific product name by the U.S. Department of Agriculture does not constitute an endorsement and does not imply a recommendation over other suitable products.

## 2D PAGE

Proteins labeled *in vivo* were prepared for isoelectric focusing by a phenol partitioning method exactly as described by Hurkman and Tanaka (9). In brief, roots of plants labeled for 3 h were rinsed with ice cold water and ground in 500  $\mu$ L of extraction buffer (0.7 M sucrose, 0.5 M Tris, 30 mM HCl, 50 mM EDTA, 0.1 M KCl, 2% [v/v] 2-mercaptoethanol). Proteins were partitioned into phenol, precipitated with ammonium acetate in methanol, rinsed with acetone, and dried under a stream of nitrogen gas. Proteins were solubilized in 100  $\mu$ L of 9 M urea, 4% (v/v) NP-40, 2% (v/v) 2-mercaptoethanol, and 2% (v/v) ampholytes (Servalytes, Serva). Translation products were prepared as above except that an equal volume of 2X extraction buffer was added to the reaction mix followed by addition of extraction buffer to a final volume of 500  $\mu$ L. This method was used to eliminate artifacts that resulted when translation products were precipitated with ethanol or methanol prior to solubilization.

2D PAGE was done according to O'Farrell (15) with the modifications of Hurkman and Tanaka (8). The first dimension isoelectric focusing gels contained pH 3 to 10 and pH 5 to 7 ampholytes (1:4, v/v) and were loaded with 200,000 cpm per gel. The second dimension SDS gels contained 10% acrylamide. Molecular weight standards (Electrophoresis Calibration Kit for mol wt determination of low mol wt proteins, Pharmacia) were dabsylated (25) and used as marker proteins on the SDS gels. Following electrophoresis of the second dimension, gels were fixed in 50% methanol and 7.5% acetic acid for 30 min. Gels were then processed for fluorography by the method of Garrels (6) and were dried.

## Computer Analysis

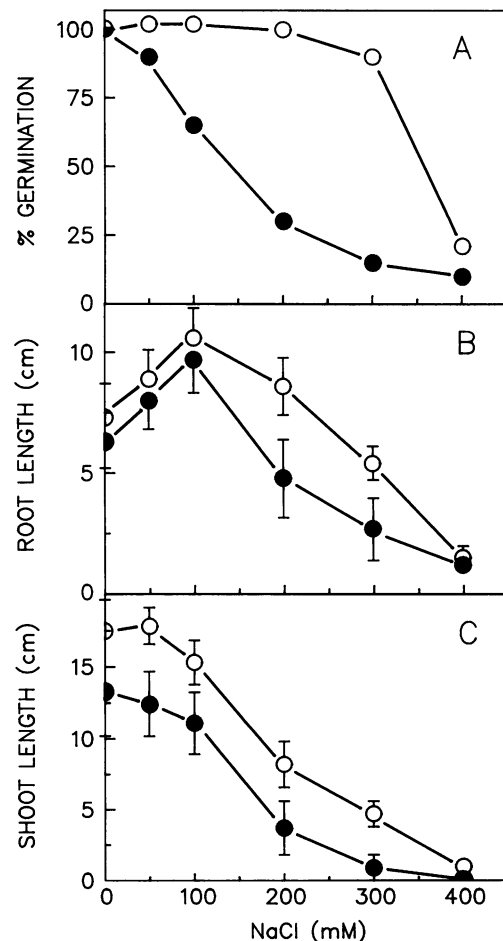
The dried gels were sent to Protein Data Bases, Inc. (Huntington Station, NY) for computer-assisted 2D gel analysis. The gels, along with calibration strips that contained known amounts of radioactive proteins (CALSTRIP), were used to expose x-ray film for 1, 3, and 8 d. The fluorographs of the calibrated multiple exposures were scanned and digitized using PDQ-SCAN, software that accesses and controls a photodiode array camera interfaced to a minicomputer. The spots were detected, the multiple exposures of the same gel merged, a composite image generated, and resultant spots matched and quantitated using PDQUEST software. A set of barley polypeptides with known  $M_r$ , based on standards, and pI, established by measurement of the first dimension pH gradient, were used by the software to calculate  $M_r$  and pI for each polypeptide. The data from the computer analyses were transferred to 5.25 inch discs for spreadsheet analysis using Quattro (Borland International), version 1.0, on an IBM compatible PC. The polypeptides discussed in this paper were obtained by spreadsheet searches in which a set of entries having 50 dpm or more in either column of a paired comparison were selected. These data sets were then searched for polypeptides that were enriched in each cultivar and for polypeptides that increased or decreased with salt treatment. Polypeptides designated as enriched in CM 72 or Prato were limited to those polypeptide spots that contained threefold or more dpm over the corresponding polypeptides of the other

cultivar. Polypeptides designated as increases or decreases with salt treatment were limited to those whose levels changed 1.5-fold or more. The data for the *in vivo* labeled polypeptides and for the translation products were treated as two different matched sets. The polypeptides detected by the spreadsheet analyses were verified by visual inspection of the fluorographs. The fluorographs reproduced in the figures are photographs of x-ray films exposed for 3 d, since, of the three film exposure times, they most clearly showed the changes detected by the computer analyses.

## RESULTS

### Comparison of Effects of NaCl on Seed Germination and Plant Growth in CM 72 and Prato

The effect of increasing concentrations of NaCl on germination of seeds of CM 72 and Prato was examined to demonstrate the difference in salt tolerance of these two cultivars (Fig. 1A). In CM 72, seed germination was not affected by NaCl concentrations of up to 200 mM, but was significantly



**Figure 1.** Comparison of the effect of salt on germination and growth of CM 72, a salt-tolerant barley cultivar, and Prato, a salt-sensitive barley cultivar. Plants were grown hydroponically in nutrient solutions containing 0 to 400 mM NaCl. After 6 d, percent seed germination relative to control (A), root length (B), and shoot length (C) were measured. CM 72, ○; Prato, ●.

reduced by 300 mM and 400 mM NaCl. Seed germination for Prato was significantly less for every NaCl concentration tested, particularly at concentrations of 100 mM and above. The effect of increasing salt concentrations on root and shoot length of CM 72 and Prato was also examined. Relative to the control, root length of both CM 72 and Prato increased when plants were grown with 50 and 100 mM NaCl and decreased when plants were grown with NaCl concentrations greater than 100 mM (Fig. 1B). Shoot length of CM 72 decreased with increasing NaCl concentrations above 50 mM. Shoot length of Prato decreased when plants were grown with increasing NaCl concentrations (Fig. 1C). A comparison of the results of root and shoot length for CM 72 and Prato showed that, despite the substantial effect of NaCl on germination of Prato seeds, the growth of Prato seeds that did germinate was similar to that of CM 72. The data indicated that 200 mM NaCl was a comparable salt stress for roots of both CM 72 and Prato at this stage of plant development.

#### Comparison of Polypeptides Labeled *in Vivo* in Control Roots of CM 72 and Prato

The fluorographs of the *in vivo* labeled polypeptides of CM 72 (Fig. 2A) and Prato (Fig. 2B) revealed that the polypeptide patterns were strikingly similar. Computer comparison of the fluorographs of CM 72 and Prato produced a matched set of 956 polypeptides. The  $M_r$  of the polypeptides ranged from 17,500 to 178,900, and pIs ranged from approximately 4.7 to 7.8. Of the many polypeptides present on the fluorographs of CM 72 and Prato, the computer analysis did not find any polypeptides that were unique to either cultivar.

Although the polypeptide patterns of CM 72 and Prato were qualitatively the same, individual polypeptides of the two cultivars differed quantitatively. To analyze these quantitative differences, a spreadsheet containing the data for the CM 72 and Prato controls was searched for polypeptides having 50 dpm or more in either cultivar. Of the 956 polypeptides in the matched set, a set of 329 polypeptides met this criterion. A small number of the polypeptides in each set were enriched threefold or more in CM 72 (21 polypeptides; Fig. 2A, Table I) or in Prato (12 polypeptides; Fig. 2B, Table I). The majority of the polypeptides enriched in CM 72 had pIs ranging from 6.4 to 7.2, while the majority of the polypeptides enriched in Prato had more acidic pIs ranging from 5.3 to 5.9.

#### Comparison of Salt-Induced Changes in *in Vivo* Labeled Polypeptides of CM 72 and Prato

Salt treatment did not induce the synthesis of any unique polypeptides or cause polypeptides to disappear in CM 72 or Prato. Salt treatment did cause increases or decreases in the levels of a large number of polypeptides in each cultivar. Computer analysis showed that 221 polypeptides increased and 328 decreased 1.5-fold or more in CM 72 and that 243 polypeptides increased and 266 decreased 1.5-fold or more in Prato. Of these changes, 60 polypeptides increased and 104 decreased in both CM 72 and Prato. Because of the numerous changes that were detected, we limited the detailed analyses of the polypeptide changes to those that were easily visible on

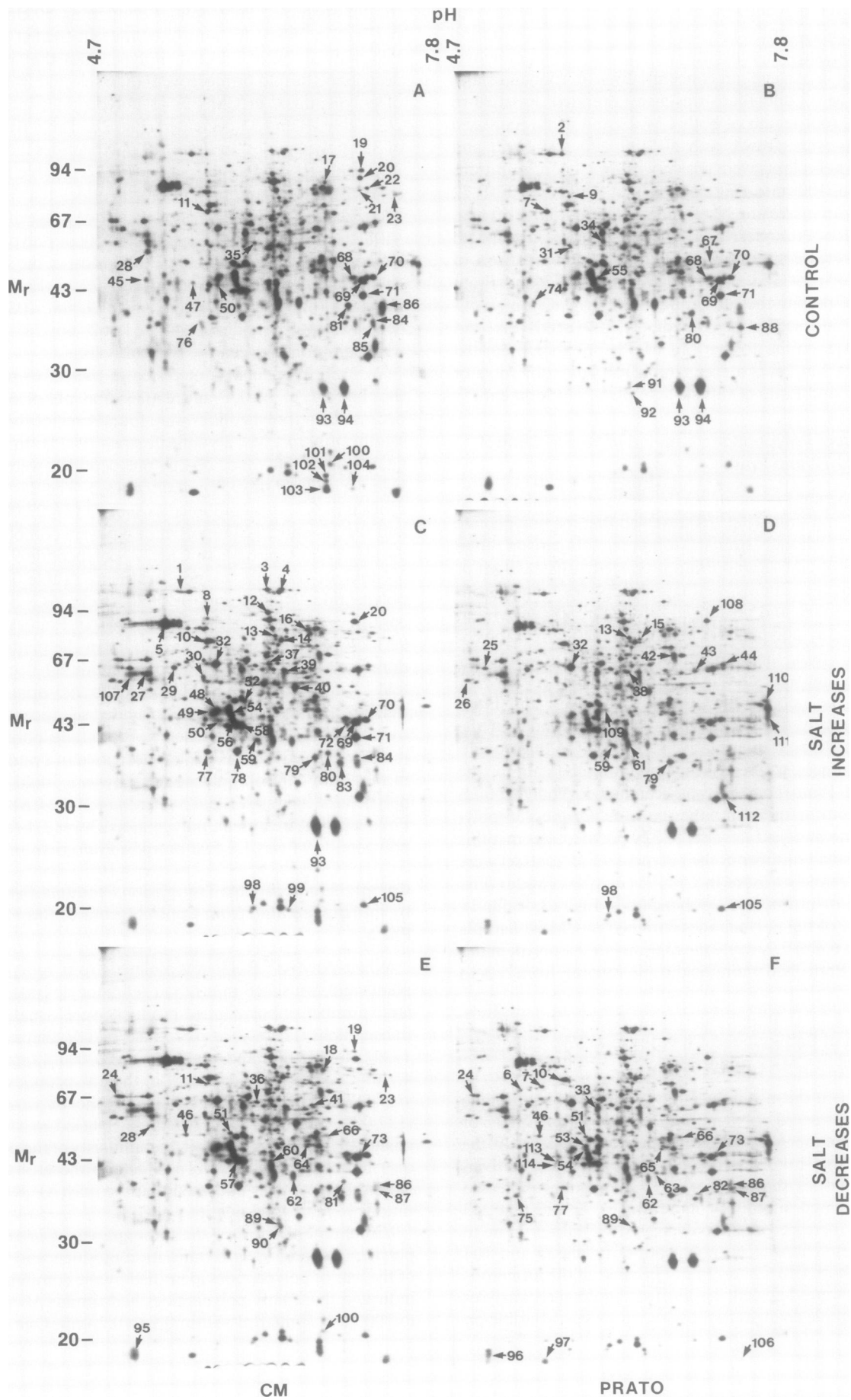
the fluorographs. Polypeptides containing 50 dpm or more per spot in either the control or the salt-treated data for the two cultivars were selected. Polypeptides that increased or decreased 1.5-fold or more were then selected from these sets of polypeptides. In CM 72, 41 polypeptides increased (Fig. 2C, Table II) and 23 decreased (Fig. 2E, Table II) out of a set of 325 polypeptides. In Prato, 19 polypeptides increased (Fig. 2D, Table III) and 25 decreased (Fig. 2F, Table III) out of a set of 311 polypeptides. The polypeptides that increased or decreased in CM 72 and Prato had a wide range of  $M_r$  (17,700–108,200) and pI (4.9–7.3); the majority of polypeptides that changed had  $M_r$  of 31,000 to 81,300. Most of the salt-induced changes were unique to either CM 72 or Prato. Only six increases (polypeptides 13, 32, 59, 79, 98, 105) and nine decreases (polypeptides 24, 46, 51, 62, 66, 73, 86, 87, 89) were common between the two cultivars.

We were particularly interested in the set of polypeptides that had  $M_r$  of 26,000 and 27,000 and pIs of 6.3 and 6.5, because these polypeptides were found previously to increase significantly in CM 72 with salt stress (9–11). In the present analysis, the 26- and 27-kD polypeptides were too close together to be resolved by the software. The 26- and 27-kD spots at pI 6.3 were combined as spot 93 ( $M_r$  27,000, pI 6.37) and the 26- and 27-kD spots at pI 6.5 were combined as spot 94 ( $M_r$  27,000, pI 6.6). In CM 72, polypeptide 93 increased 3.4-fold in response to salt treatment (Table II), whereas polypeptide 94 increased 1.2-fold (from 612 dpm in the control to 819 dpm in the salt treated). In Prato, polypeptide 93 increased 1.3-fold (from 873 dpm in the control to 1050 dpm in the salt treated) and polypeptide 94 decreased 1.4-fold (from 1404 dpm in the control to 999 dpm in the salt treated). Thus, polypeptide 93 increased significantly with salt treatment in CM 72, but not in Prato, and polypeptide 94 increased slightly in CM 72, but decreased in Prato. However, in the Prato control, polypeptide 93 was 2.5-fold higher and polypeptide 94 was 2.3-fold higher than in the CM 72 control. Following salt treatment, polypeptide 93 was 1.4-fold less and polypeptide 94 was 1.2-fold more in Prato than in CM 72.

#### Comparison of *in Vitro* Translation Products Synthesized by mRNAs Isolated from Control Roots of CM 72 and Prato

When poly(A)<sup>+</sup> RNA was isolated from roots of control plants and translated in a rabbit reticulocyte lysate system, a large number of polypeptide products were visualized on the fluorographs. These *in vitro* products had  $M_r$  that ranged from 17,800 to 132,000 and pIs that ranged from approximately 4.7 to 7.8. The upper  $M_r$  range of the polypeptides labeled *in vivo* (179,800) was greater than for the *in vitro* products (132,000), but only 15 polypeptides labeled *in vivo* had  $M_r$  greater than 132,000. Overall, the similarity of the polypeptide distributions of the *in vitro* products to those of the *in vivo* labeled polypeptides indicated that the poly(A)<sup>+</sup> RNA preparations were of good quality.

Visual comparisons of the fluorographs of *in vivo* labeled polypeptides and *in vitro* translation products and inspection of the lists of polypeptides identified by the computer analysis indicated that many polypeptides had similar  $M_r$  within 500 to 1000 and similar pI within 0.1 to 0.2 unit. One noticeable



**Table I.** Polypeptides (PP) Enriched Threefold or More in CM 72 or Prato

Polypeptides in roots of 6 d old control plants were labeled *in vivo*, separated on 2D gels, and data obtained by computer analysis of the fluorographs. Polypeptides are those indicated on the fluorographs depicted in Figure 2, A and B.

| Polypeptides Enriched in CM 72 |                         |      |            |       | Polypeptides Enriched in Prato |                         |      |            |       |
|--------------------------------|-------------------------|------|------------|-------|--------------------------------|-------------------------|------|------------|-------|
| PP No.                         | $M_r$ ( $\times 1000$ ) | pI   | CM 72      | Prato | PP No.                         | $M_r$ ( $\times 1000$ ) | pI   | CM 72      | Prato |
|                                |                         |      | <i>dpm</i> |       |                                |                         |      | <i>dpm</i> |       |
| 11                             | 68.4                    | 5.46 | 172        | 49    | 2                              | 107.0                   | 5.46 | 47         | 139   |
| 17                             | 83.7                    | 6.40 | 152        | 49    | 7                              | 68.1                    | 5.38 | 7          | 196   |
| 19                             | 90.0                    | 6.96 | 124        | 3     | 9                              | 77.3                    | 5.49 | 4          | 55    |
| 20                             | 85.5                    | 6.96 | 136        | 35    | 31                             | 51.1                    | 5.46 | 5          | 59    |
| 21                             | 78.6                    | 6.91 | 56         | 3     | 34                             | 53.6                    | 5.65 | 29         | 92    |
| 22                             | 79.6                    | 7.09 | 79         | 3     | 55                             | 42.9                    | 5.60 | 4          | 85    |
| 23                             | 75.6                    | 7.42 | 100        | 3     | 67                             | 45.8                    | 6.77 | 42         | 206   |
| 28                             | 52.0                    | 5.18 | 170        | 49    | 74                             | 39.8                    | 5.33 | 4          | 72    |
| 35                             | 53.5                    | 5.70 | 83         | 3     | 80                             | 37.2                    | 6.51 | 39         | 202   |
| 45                             | 43.3                    | 5.16 | 70         | 23    | 88                             | 34.9                    | 7.30 | 70         | 134   |
| 47                             | 42.4                    | 5.60 | 61         | 3     | 91                             | 27.8                    | 5.93 | 22         | 69    |
| 50                             | 42.0                    | 5.49 | 95         | 17    | 92                             | 26.1                    | 5.93 | 4          | 83    |
| 76                             | 36.1                    | 5.43 | 52         | 3     |                                |                         |      |            |       |
| 81                             | 38.6                    | 6.66 | 229        | 3     |                                |                         |      |            |       |
| 84                             | 35.7                    | 7.17 | 103        | 47    |                                |                         |      |            |       |
| 85                             | 35.0                    | 7.19 | 69         | 13    |                                |                         |      |            |       |
| 100                            | 20.4                    | 6.40 | 120        | 29    |                                |                         |      |            |       |
| 101                            | 19.1                    | 6.37 | 159        | 3     |                                |                         |      |            |       |
| 102                            | 18.4                    | 6.37 | 129        | 3     |                                |                         |      |            |       |
| 103                            | 17.9                    | 6.37 | 327        | 4     |                                |                         |      |            |       |
| 104                            | 18.0                    | 6.67 | 76         | 3     |                                |                         |      |            |       |

group of polypeptides had similar  $M_r$  and pI *in vivo* and *in vitro*. The *in vivo* polypeptides in this group included 68 ( $M_r$  42,500, pI 6.71), 69 ( $M_r$  42,100, pI 6.94), 70 ( $M_r$  42,500, pI 7.20), and 71 ( $M_r$  40,100, pI 6.94) (Fig. 2) and the corresponding *in vitro* polypeptides were 34 ( $M_r$  42,900, pI 6.78), 35 ( $M_r$  42,000, pI 6.86), 36 ( $M_r$  42,500, pI 7.00), and 37 ( $M_r$  39,100, pI 6.87) (Fig. 3). Polypeptides synthesized *in vitro* that had  $M_r$  and pI that exactly corresponded to those of polypeptides 93 and 94 were not found. Because of their mobilities, it is tempting to speculate that the *in vitro* polypeptide products 54 and 55 may be related to the *in vivo* polypeptides 93 and 94.

Computer comparison of the fluorographs of the *in vitro* products of CM 72 and Prato produced a matched set of 863 polypeptide spots. This number was 10% less than that observed for the *in vivo* labeled polypeptides and is probably a reflection of the inability of the rabbit reticulocyte lysate system to translate all of the poly(A)<sup>+</sup> RNAs. Fewer products

were revealed on fluorographs of 2D gels when the poly(A)<sup>+</sup> RNA was translated in a wheat germ system (NL Robinson, CK Tanaka, WJ Hurkman, unpublished observations).

The 2D patterns for the *in vitro* translation products of CM 72 (Fig. 3A) and Prato (Fig. 3B) were qualitatively the same; the computer analysis did not find any polypeptides that were unique to either cultivar. Individual polypeptides did differ quantitatively between the two cultivars. A set of polypeptides containing 50 dpm or more per spot in either CM 72 or Prato was computer-selected from the data for controls in the matched set to assess these quantitative differences. Of the 863 polypeptide spots in the matched set, a set of 188 polypeptides met this criterion. Fourteen polypeptides in CM 72 (Fig. 3A, Table IV) and only two (polypeptides 26 and 51) in Prato (Fig. 3B, Table IV) were enriched threefold or more. The majority of the polypeptides enriched in CM 72 had pIs of 6.3 to 7.0, a finding consistent with that observed for the distribution of polypeptides that were enriched *in vivo*.

**Figure 2.** Comparison of the effect of salt on the *in vivo* labeling of polypeptides in roots of CM 72, a salt-tolerant barley cultivar, and Prato, a salt-sensitive barley cultivar. A, Fluorograph of polypeptides of roots of control plants of CM 72. The arrows indicate polypeptides listed in Table I that were enriched threefold or more over Prato. B, Fluorograph of polypeptides of roots of control plants of Prato. The arrows indicate polypeptides listed in Table I that were enriched threefold or more over CM 72. Polypeptides 68–71, which are not enriched in CM 72 or Prato and also indicated on A and B, may correspond to polypeptides 34–37 in Figure 3, A and B. Similarly, polypeptides 93 and 94 may correspond to polypeptides 54 and 55 in Figure 3, A and B. C, Fluorograph of polypeptides of roots of CM 72 treated with 200 mM NaCl. The arrows indicate polypeptides listed in Table II that increased with salt stress. D, Fluorograph of polypeptides of roots of Prato treated with 200 mM NaCl. The arrows indicate polypeptides listed in Table III that increased with salt. E, The same fluorograph as in D, except that the arrows indicate polypeptides listed in Table II that decreased with salt stress in CM 72. F, The same fluorograph as in D, except that the arrows indicate polypeptides listed in Table III that decreased with salt stress in Prato.

**Table II.** Polypeptides (PP) That Increase or Decrease 1.5-fold or More in CM 72 when Plants are Grown in the Presence of 200 mM NaCl for 6 d

Polypeptides in roots of control and salt treated plants were labeled *in vivo*, separated on 2D gels, and data obtained by computer analysis of the fluorographs. Polypeptides are those indicated on the fluorographs depicted in Figure 2, C and E.

| Polypeptides That Increase |                         |      |       |       | Polypeptides That Decrease |                         |      |       |       |
|----------------------------|-------------------------|------|-------|-------|----------------------------|-------------------------|------|-------|-------|
| PP No.                     | $M_r$ ( $\times 1000$ ) | pI   | CM 72 | Prato | PP No.                     | $M_r$ ( $\times 1000$ ) | pI   | CM 72 | Prato |
| <i>dpm</i>                 |                         |      |       |       | <i>dpm</i>                 |                         |      |       |       |
| 1                          | 108.2                   | 5.35 | 76    | 159   | 11                         | 68.4                    | 5.46 | 172   | 28    |
| 3                          | 108.7                   | 5.90 | 97    | 155   | 18                         | 78.3                    | 6.45 | 203   | 117   |
| 4                          | 109.5                   | 6.05 | 105   | 170   | 19                         | 90.0                    | 6.96 | 124   | 64    |
| 5                          | 81.3                    | 5.26 | 279   | 886   | 23                         | 75.6                    | 7.42 | 100   | 53    |
| 8                          | 85.5                    | 5.47 | 51    | 78    | 24                         | 65.3                    | 4.89 | 97    | 60    |
| 10                         | 71.7                    | 5.47 | 189   | 547   | 28                         | 52.0                    | 5.18 | 170   | 95    |
| 12                         | 88.7                    | 5.91 | 118   | 204   | 36                         | 59.5                    | 5.76 | 144   | 89    |
| 13                         | 75.1                    | 5.97 | 64    | 102   | 41                         | 59.1                    | 6.33 | 222   | 96    |
| 14                         | 72.9                    | 6.04 | 99    | 155   | 46                         | 48.3                    | 5.37 | 126   | 71    |
| 16                         | 80.0                    | 6.30 | 71    | 167   | 57                         | 41.3                    | 5.59 | 205   | 117   |
| 20                         | 85.5                    | 6.92 | 136   | 384   | 60                         | 42.1                    | 5.92 | 200   | 74    |
| 27                         | 57.7                    | 5.18 | 189   | 395   | 62                         | 39.1                    | 6.16 | 258   | 171   |
| 29                         | 60.0                    | 5.31 | 82    | 146   | 64                         | 45.0                    | 6.31 | 105   | 62    |
| 30                         | 54.1                    | 5.45 | 74    | 139   | 66                         | 47.8                    | 6.50 | 165   | 64    |
| 32                         | 60.0                    | 5.50 | 290   | 678   | 73                         | 42.4                    | 7.03 | 208   | 4     |
| 37                         | 60.6                    | 5.91 | 62    | 110   | 81                         | 38.6                    | 6.66 | 229   | 72    |
| 39                         | 57.7                    | 6.07 | 95    | 159   | 86                         | 38.0                    | 7.30 | 290   | 121   |
| 40                         | 52.0                    | 6.20 | 127   | 305   | 87                         | 37.0                    | 7.30 | 158   | 66    |
| 48                         | 43.9                    | 5.50 | 136   | 227   | 89                         | 31.5                    | 5.99 | 116   | 61    |
| 49                         | 42.9                    | 5.49 | 150   | 283   | 90                         | 31.0                    | 6.01 | 149   | 72    |
| 50                         | 42.0                    | 5.49 | 95    | 227   | 95                         | 18.4                    | 5.00 | 176   | 115   |
| 52                         | 46.9                    | 5.65 | 73    | 134   | 100                        | 20.4                    | 6.40 | 120   | 55    |
| 54                         | 42.9                    | 5.57 | 226   | 414   |                            |                         |      |       |       |
| 56                         | 41.9                    | 5.58 | 189   | 336   |                            |                         |      |       |       |
| 58                         | 41.3                    | 5.63 | 288   | 645   |                            |                         |      |       |       |
| 59                         | 38.9                    | 5.76 | 83    | 189   |                            |                         |      |       |       |
| 69                         | 42.1                    | 6.94 | 404   | 676   |                            |                         |      |       |       |
| 70                         | 42.5                    | 7.20 | 254   | 418   |                            |                         |      |       |       |
| 71                         | 40.1                    | 6.94 | 393   | 718   |                            |                         |      |       |       |
| 72                         | 41.9                    | 6.77 | 94    | 168   |                            |                         |      |       |       |
| 77                         | 37.0                    | 5.47 | 146   | 223   |                            |                         |      |       |       |
| 78                         | 37.0                    | 5.60 | 319   | 549   |                            |                         |      |       |       |
| 79                         | 36.3                    | 6.36 | 55    | 207   |                            |                         |      |       |       |
| 83                         | 35.7                    | 6.62 | 88    | 831   |                            |                         |      |       |       |
| 84                         | 35.7                    | 7.17 | 103   | 170   |                            |                         |      |       |       |
| 93                         | 27.0                    | 6.37 | 318   | 1044  |                            |                         |      |       |       |
| 98                         | 19.4                    | 5.70 | 71    | 128   |                            |                         |      |       |       |
| 99                         | 19.2                    | 6.10 | 82    | 132   |                            |                         |      |       |       |
| 105                        | 20.1                    | 7.11 | 207   | 313   |                            |                         |      |       |       |
| 107                        | 57.8                    | 4.99 | 4     | 224   |                            |                         |      |       |       |

#### Comparison of *In Vitro* Translation Products Synthesized by mRNAs Isolated from Salt-Treated Roots of CM 72 and Prato

When poly(A)<sup>+</sup> RNA was isolated from roots of salt-grown plants of CM 72 and Prato and translated in a rabbit reticulocyte cell free system, it was found that the population of translatable mRNAs was altered by salt treatment. Computer analysis of the translation products showed that a large number of polypeptides increased or decreased in each cultivar, but none were unique or disappeared. In CM 72, 142 polypeptides increased and 467 decreased 1.5-fold or more and in

Prato 305 polypeptides increased and 217 decreased 1.5-fold or more. Of these changes, 47 polypeptides increased and 121 decreased in both CM 72 and Prato. As was done for the *in vivo* comparisons, the analysis of the *in vitro* products was limited to those polypeptides that contained 50 dpm or more per spot in either the control or salt-treated data and that increased or decreased 1.5-fold or more. In CM 72, 10 polypeptides increased (Fig. 3C, Table V) and 28 decreased (Fig. 3E, Table V) out of a set of 169 polypeptides. In Prato, 14 polypeptides increased (Fig. 3D, Table VI) and 17 decreased (Fig. 3F, Table VI) out of a set of 179 polypeptides. The polypeptides that increased or decreased in CM 72 and Prato



**Table III.** Polypeptides (PP) That Increase or Decrease 1.5-fold or More in Prato When Plants Were Grown in the Presence of 200 mM NaCl for 6 d

Polypeptides in roots of control and salt treated plants were labeled *in vivo*, separated on 2D gels, and data obtained by computer analysis of the fluorographs. Polypeptides are those indicated on the fluorographs depicted in Figure 2, D and F.

| Polypeptides That Increase |                         |      |            |       | Polypeptides That Decrease |                         |      |            |       |
|----------------------------|-------------------------|------|------------|-------|----------------------------|-------------------------|------|------------|-------|
| PP No.                     | $M_r$ ( $\times 1000$ ) | pI   | CM 72      | Prato | PP No.                     | $M_r$ ( $\times 1000$ ) | pI   | CM 72      | Prato |
|                            |                         |      | <i>dpm</i> |       |                            |                         |      | <i>dpm</i> |       |
| 13                         | 75.1                    | 5.97 | 64         | 98    | 6                          | 67.4                    | 5.27 | 75         | 50    |
| 15                         | 75.1                    | 6.09 | 51         | 78    | 7                          | 68.1                    | 5.38 | 196        | 85    |
| 25                         | 60.8                    | 4.98 | 66         | 114   | 10                         | 71.7                    | 5.47 | 291        | 60    |
| 26                         | 54.8                    | 4.67 | 61         | 101   | 24                         | 65.3                    | 4.89 | 90         | 60    |
| 32                         | 60.0                    | 5.50 | 430        | 688   | 33                         | 57.4                    | 5.63 | 267        | 172   |
| 38                         | 58.1                    | 5.94 | 60         | 114   | 46                         | 48.3                    | 5.37 | 114        | 69    |
| 42                         | 66.4                    | 6.42 | 78         | 221   | 51                         | 47.6                    | 5.57 | 162        | 67    |
| 43                         | 59.1                    | 6.66 | 64         | 112   | 53                         | 44.5                    | 5.57 | 437        | 150   |
| 44                         | 61.3                    | 7.24 | 84         | 126   | 54                         | 42.9                    | 5.57 | 508        | 174   |
| 59                         | 38.9                    | 5.76 | 67         | 147   | 62                         | 39.1                    | 6.16 | 374        | 113   |
| 61                         | 40.8                    | 5.92 | 103        | 216   | 63                         | 40.3                    | 6.25 | 124        | 55    |
| 79                         | 36.3                    | 6.36 | 102        | 224   | 65                         | 45.1                    | 6.35 | 117        | 50    |
| 98                         | 19.4                    | 5.70 | 52         | 101   | 66                         | 47.8                    | 6.50 | 119        | 59    |
| 105                        | 20.1                    | 7.11 | 152        | 242   | 73                         | 42.4                    | 7.03 | 174        | 70    |
| 108                        | 85.5                    | 6.92 | 35         | 211   | 75                         | 36.2                    | 5.23 | 153        | 102   |
| 109                        | 44.5                    | 5.65 | 48         | 206   | 77                         | 37.0                    | 5.47 | 220        | 113   |
| 110                        | 48.1                    | 7.58 | 3          | 162   | 82                         | 36.5                    | 6.65 | 108        | 59    |
| 111                        | 45.7                    | 7.58 | 3          | 421   | 86                         | 38.0                    | 7.30 | 121        | 68    |
| 112                        | 31.1                    | 7.22 | 3          | 125   | 87                         | 37.0                    | 7.30 | 179        | 106   |
|                            |                         |      |            |       | 89                         | 31.5                    | 5.99 | 105        | 66    |
|                            |                         |      |            |       | 96                         | 18.0                    | 5.01 | 220        | 88    |
|                            |                         |      |            |       | 97                         | 18.0                    | 5.38 | 298        | 137   |
|                            |                         |      |            |       | 106                        | 17.7                    | 7.42 | 280        | 51    |
|                            |                         |      |            |       | 113                        | 42.1                    | 5.46 | 123        | 20    |
|                            |                         |      |            |       | 114                        | 41.5                    | 5.45 | 118        | 49    |

had a wide range of  $M_r$  (17,800–103,700) and pI (5.2–6.9); the majority of polypeptides that changes had  $M_r$  of 30,500 to 82,300. Of these changes, the majority were unique to each cultivar. Only one increase (polypeptide 40) and eight decreases (polypeptides 7, 19, 23–25, 31, 32, 56) were common to the two cultivars.

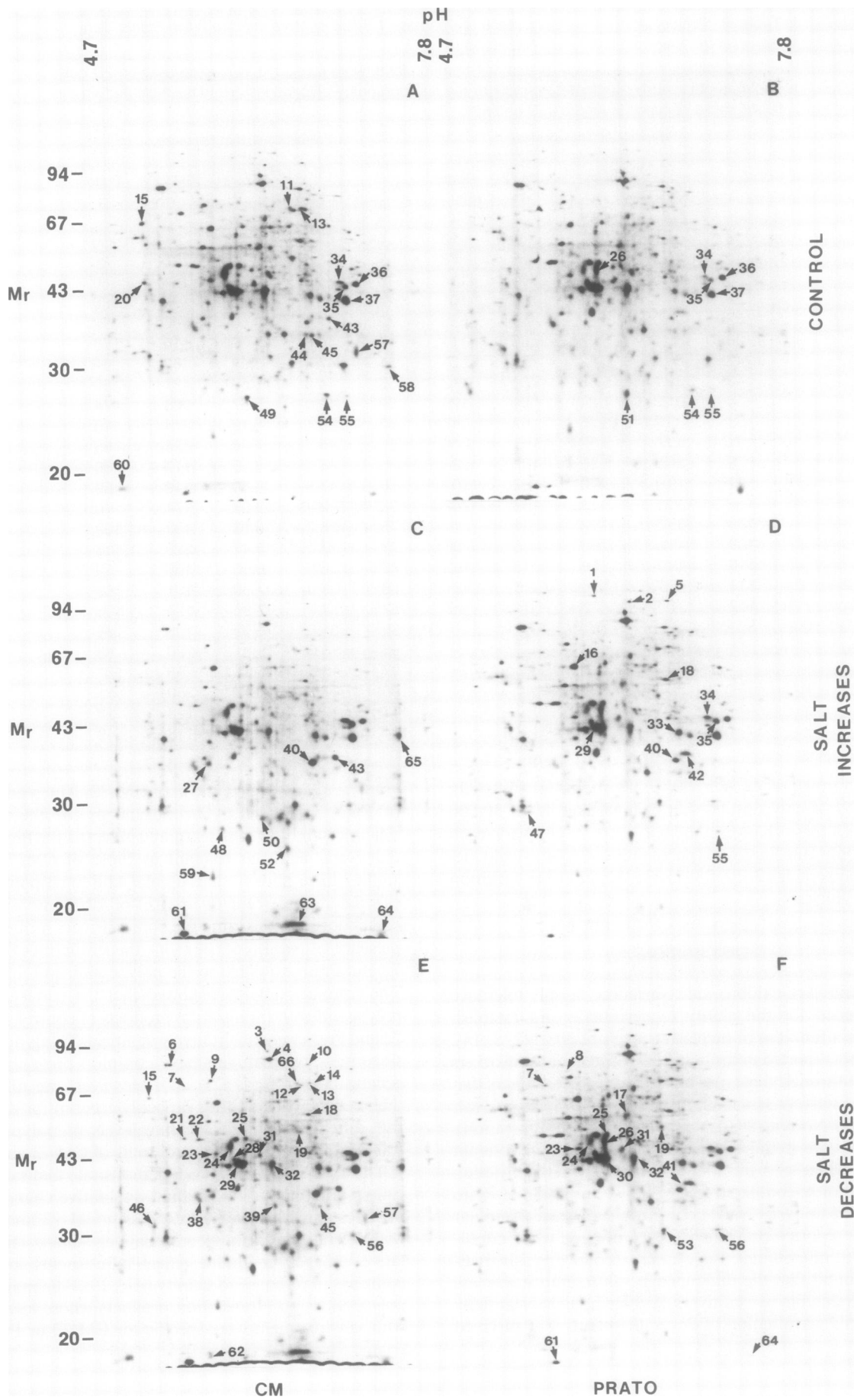
## DISCUSSION

Barley cultivars exhibit wide variation in salt tolerance when screened for the ability to germinate and establish seedlings as a function of increasing levels of NaCl (2, 3, 14). Although not reported in the literature, Epstein and coworkers have found through screening numerous barley cultivars that CM 72 is relatively salt tolerant and Prato is relatively salt sensitive (JD Norlyn, UC-Davis, personal communication). In this study, we found that CM 72 is indeed more tolerant than Prato when seed germination was used as the criterion for salt tolerance; compared to CM 72, germination of Prato was inhibited at every NaCl concentration tested. A similar finding was reported previously (18). However, when cumulative growth of seedlings was examined following salt treatment, we found that the difference in salt tolerance was less striking between the two cultivars. Although salt substantially inhibited seed germination of Prato, the plants that survived

grew nearly as well as those of CM 72. The seed population of Prato is apparently heterogeneous for the trait of salt tolerance and the salt treatments selected for Prato seedlings that were nearly as tolerant as those of CM 72. Previous studies have shown that uptake and incorporation (when calculated as percent of uptake) of label into excised roots and shoots was the same for both cultivars in the absence or presence of NaCl (16). In roots, the similarities between CM 72 and Prato extended to the polypeptide and mRNA levels. The computer analyses showed that the patterns of labeled polypeptides and translation products of control roots of CM 72 and Prato were qualitatively identical. This is not surprising since these two cultivars have common progenitors in their genetic backgrounds (20, 21). However, the differences in their genetic backgrounds probably account for the quantitative differences in the labeling levels of polypeptides and translation products of control roots of CM 72 and Prato.

The analyses by 2D PAGE demonstrate that salt treatment causes quantitative rather than qualitative changes in polypeptides and translatable mRNAs in CM 72 and Prato and that, overall, the changes are relatively small. These quantitative changes no doubt reflect adjustments in metabolic pathways in response to salinity (*e.g.*, 19). The majority of the polypeptides and translation products whose levels changed with salt treatment were specific for CM 72 or Prato. Based





**Table IV.** Polypeptide (PP) Products Enriched Threefold or More in CM 72 or Prato

Poly(A)<sup>+</sup> RNA was isolated from roots of 6 d old control plants and translated *in vitro* using a rabbit reticulocyte lysate system. The products were separated on 2D gels and data obtained by computer analysis of the fluorographs. Polypeptides are those indicated on the fluorographs depicted in Figure 3, A and B.

| Polypeptides Enriched in CM 72 |                              |      |            |       | Polypeptides Enriched in Prato |                              |      |            |       |
|--------------------------------|------------------------------|------|------------|-------|--------------------------------|------------------------------|------|------------|-------|
| PP No.                         | <i>M<sub>r</sub></i> (×1000) | pI   | CM 72      | Prato | PP No.                         | <i>M<sub>r</sub></i> (×1000) | pI   | CM 72      | Prato |
|                                |                              |      | <i>dpm</i> |       |                                |                              |      | <i>dpm</i> |       |
| 11                             | 70.3                         | 6.32 | 76         | 20    | 26                             | 45.3                         | 5.75 | 42         | 172   |
| 13                             | 70.0                         | 6.37 | 148        | 42    | 51                             | 26.6                         | 6.09 | 4          | 151   |
| 15                             | 63.0                         | 5.15 | 127        | 43    |                                |                              |      |            |       |
| 20                             | 43.5                         | 5.16 | 84         | 24    |                                |                              |      |            |       |
| 34                             | 42.9                         | 6.78 | 238        | 80    |                                |                              |      |            |       |
| 35                             | 42.0                         | 6.86 | 137        | 248   |                                |                              |      |            |       |
| 43                             | 36.7                         | 6.63 | 96         | 17    |                                |                              |      |            |       |
| 44                             | 34.7                         | 6.36 | 60         | 17    |                                |                              |      |            |       |
| 45                             | 34.4                         | 6.41 | 98         | 30    |                                |                              |      |            |       |
| 49                             | 26.7                         | 5.80 | 148        | 14    |                                |                              |      |            |       |
| 55                             | 26.8                         | 6.86 | 77         | 15    |                                |                              |      |            |       |
| 57                             | 32.1                         | 7.00 | 140        | 24    |                                |                              |      |            |       |
| 58                             | 30.3                         | 7.30 | 74         | 18    |                                |                              |      |            |       |
| 60                             | 18.4                         | 5.00 | 246        | 30    |                                |                              |      |            |       |

on the differences in their genetic backgrounds, this phenomenon is probably due to variability in the response of each of the cultivars to salt. Because the seedlings of CM 72 and Prato have similar tolerance to salt, whether or not specific changes at the polypeptide and translatable mRNA levels are related to the trait of salt tolerance in barley could not be determined. Age and water status of the roots may be complicating factors in the interpretation of the data for salt stress. Salt treatment could alter the physiological age of the two cultivars and the observed changes could be due, in part, to differences in developmental states. The possibility that the specificity of the responses of the two cultivars to salt could be due to differences in intracellular water potentials cannot be ruled out.

Previous reports (16, 17) on the effects of NaCl on polypeptide changes in roots of CM 72 and Prato differ from our results. In an *in vivo* labeling experiment, Ramagopal (17) found that the levels of relatively few polypeptides changed with salt treatment and that the same changes occurred in both CM 72 and Prato (the data for Prato was not shown). A set of six new polypeptides were also induced by salt in both

cultivars; this set of polypeptides included polypeptides R1–R4 (see ref. 17, Fig. 1), which correspond to polypeptides 93 and 94 in this paper and polypeptides S1–S4 in previous papers (10, 11). In contrast, our results show a far greater number of salt-induced changes in CM 72 and Prato. The majority of changes were cultivar specific and no unique polypeptides were observed. In CM 72, we found, in this study and previous studies (9–11), that polypeptides 93 and 94 increased with salt treatment, but were not newly induced. Furthermore, we found that they were not newly induced and did not increase with salt in Prato. Ramagopal (16) reported that the levels of relatively few translation products changed with salt treatment. Analysis of these products showed that the patterns of CM 72 and Prato were qualitatively different and, further, that different products were newly induced by salt in CM 72 and in Prato (16). In contrast, our results show that the translation products of CM 72 and Prato poly(A)<sup>+</sup> RNAs were qualitatively the same. In addition, a far greater number of salt-induced changes in the translation products were observed in CM 72 and Prato (Fig. 3), where the majority of changes were cultivar specific. No unique salt-induced

**Figure 3.** Comparison of polypeptide products synthesized *in vitro* by mRNAs isolated from control and salt-treated roots of CM 72, a salt-sensitive barley cultivar, and Prato, a salt-tolerant barley cultivar. A, Fluorograph of polypeptide products synthesized by mRNAs isolated from roots of control plants of CM 72. The arrows indicate polypeptides listed in Table IV that were enriched threefold or more over Prato. B, Fluorograph of polypeptide products synthesized by mRNAs isolated from roots of control plants of Prato. The arrows indicate polypeptides listed in Table IV that were enriched threefold or more over CM 72. Polypeptides 34–37, indicated on both A and B, may correspond to polypeptides 68–71 in Figure 2, A and B. Polypeptides 34 and 35 were not enriched in either CM 72 or Prato. Similarly, polypeptides 54 and 55 may correspond to polypeptides 93 and 94 in Figure 2, A and B. Polypeptide 55 was enriched in CM 72. C, Fluorograph of polypeptide products synthesized by mRNAs isolated from roots of CM 72 treated with 200 mM NaCl. The arrows indicate polypeptides listed in Table V that increased with salt stress. D, Fluorograph of polypeptide products synthesized by mRNAs isolated from roots of Prato treated with 200 mM NaCl. The arrows indicate polypeptides listed in Table VI that increased with salt. E, The same fluorograph as in C, except that the arrows indicate polypeptides listed in Table V that decreased with salt stress in CM 72. F, The same fluorograph as in D, except that the arrows indicate polypeptides listed in Table VI that decreased with salt stress in Prato.

**Table V.** Polypeptide (PP) Products That Increase or Decrease 1.5-fold or More in CM 72

Poly(A)<sup>+</sup> RNA was isolated from roots of 6 d old plants grown in the presence or absence of 200 mM NaCl and translated *in vitro* using a rabbit reticulocyte lysate system. The products were separated on 2D gels and data obtained by computer analysis of the fluorographs. Polypeptides are those indicated on the fluorographs depicted in Figure 3, C and D.

| Polypeptides That Increase |                         |      |            |       | Polypeptides That Decrease |                         |      |            |       |
|----------------------------|-------------------------|------|------------|-------|----------------------------|-------------------------|------|------------|-------|
| PP No.                     | $M_r$ ( $\times 1000$ ) | pI   | CM 72      | Prato | PP No.                     | $M_r$ ( $\times 1000$ ) | pI   | CM 72      | Prato |
|                            |                         |      | <i>dpm</i> |       |                            |                         |      | <i>dpm</i> |       |
| 40                         | 36.2                    | 6.38 | 92         | 326   | 3                          | 90.9                    | 6.01 | 98         | 36    |
| 43                         | 36.7                    | 6.63 | 96         | 192   | 4                          | 85.4                    | 6.03 | 294        | 169   |
| 48                         | 27.7                    | 5.65 | 52         | 82    | 6                          | 83.0                    | 5.30 | 210        | 59    |
| 50                         | 29.2                    | 5.99 | 52         | 89    | 7                          | 68.1                    | 5.39 | 111        | 4     |
| 52                         | 25.4                    | 6.27 | 62         | 99    | 9                          | 72.8                    | 5.55 | 118        | 30    |
| 59                         | 22.6                    | 5.61 | 80         | 124   | 10                         | 82.3                    | 6.36 | 103        | 21    |
| 61                         | 18.0                    | 5.41 | 176        | 377   | 12                         | 69.3                    | 6.33 | 87         | 32    |
| 63                         | 18.8                    | 6.06 | 77         | 139   | 13                         | 70.0                    | 6.37 | 148        | 32    |
| 64                         | 17.8                    | 7.34 | 198        | 694   | 14                         | 69.6                    | 6.38 | 153        | 4     |
| 65                         | 39.6                    | 7.67 | 4          | 121   | 15                         | 63.0                    | 5.15 | 127        | 43    |
|                            |                         |      |            |       | 18                         | 55.0                    | 6.36 | 135        | 54    |
|                            |                         |      |            |       | 19                         | 50.1                    | 6.32 | 134        | 64    |
|                            |                         |      |            |       | 21                         | 48.3                    | 5.38 | 110        | 42    |
|                            |                         |      |            |       | 22                         | 48.0                    | 5.48 | 102        | 62    |
|                            |                         |      |            |       | 23                         | 43.8                    | 5.59 | 117        | 65    |
|                            |                         |      |            |       | 24                         | 45.9                    | 5.67 | 316        | 199   |
|                            |                         |      |            |       | 25                         | 48.0                    | 5.75 | 129        | 81    |
|                            |                         |      |            |       | 28                         | 42.3                    | 5.69 | 153        | 98    |
|                            |                         |      |            |       | 29                         | 41.3                    | 5.71 | 261        | 173   |
|                            |                         |      |            |       | 31                         | 42.8                    | 5.90 | 153        | 64    |
|                            |                         |      |            |       | 32                         | 41.4                    | 6.06 | 311        | 157   |
|                            |                         |      |            |       | 38                         | 35.6                    | 5.47 | 141        | 50    |
|                            |                         |      |            |       | 39                         | 34.6                    | 6.08 | 100        | 54    |
|                            |                         |      |            |       | 45                         | 34.4                    | 6.41 | 98         | 33    |
|                            |                         |      |            |       | 46                         | 32.0                    | 5.20 | 98         | 60    |
|                            |                         |      |            |       | 56                         | 30.5                    | 6.83 | 269        | 107   |
|                            |                         |      |            |       | 57                         | 32.1                    | 7.00 | 140        | 57    |
|                            |                         |      |            |       | 62                         | 18.3                    | 5.56 | 75         | 50    |

translation products were found for either cultivar. The differences in experimental conditions—the age of plants, concentration of salt used, length of time of salt treatment, growth of plants in calcium or in a more complex nutrient solution, labeling of excised tissue pieces or intact plants, quality of fluorographs—may account for some of the discrepancies between these studies (WJ Hurkman, CK Tanaka, unpublished observations).

The response of plants to salt is complex and involves changes in growth, water relations, ion transport, and cell metabolism (1, 5, 7, 12, 13). Although the underlying mechanisms for salt tolerance remain elusive, the increases in certain polypeptides during salt stress could be important in the adaptation of plants to saline conditions. For example, a 26-kD polypeptide that increases with adaptation of cultured tobacco cells to medium containing high levels of NaCl may have a role in osmoregulation (22–24). We have shown in previous studies of CM 72 that four polypeptides with  $M_r$  of 26,000 and 27,000 and pI of 6.3 and 6.5, which are not immunologically related to osmotin (9), increased with salt treatment (9–11), decreased when plants were subsequently transferred to nutrient solution without salt (9), and did not

increase with water deficit (10) or heat shock (WJ Hurkman, CK Tanaka, unpublished observations). Computer analyses showed that the pI 6.3 and 6.5 polypeptides increased in CM 72, but not in Prato with salt treatment. In future studies, high resolution 2D gels will be used to isolate these polypeptides for antibody production and gas phase microsequencing. The antibodies and the microsequence information can be used in procedures designed to isolate the genes that correspond to these polypeptides. The characterization of these genes will provide an opportunity to more accurately assess their expression during salt stress.

In this study, we have shown that the *in vivo* labeling of polypeptides is altered by salt stress. By assaying poly(A)<sup>+</sup> RNA in an *in vitro* translation system, we have also shown that the levels of translatable mRNAs change with salt treatment. The results indicate that gene regulation is altered by salt stress, but the specific mechanisms involved are not known. A problem with the *in vitro* data is that until steady state levels of mRNAs are assayed by use of cDNA probes, we cannot say that the effects of salt on the levels of translation products accurately reflect what is occurring *in vivo*. In addition, information obtained using cDNA probes in RNA hy-

**Table VI.** Polypeptide (PP) Products That Increase or Decrease 1.5-fold or More in Prato

Poly (A)<sup>+</sup> RNA was isolated from roots of 6 d old plants grown in the presence or absence of 200 mM NaCl and translated *in vitro* using a rabbit reticulocyte lysate system. The products were separated on 2D gels and data obtained by computer analysis of the fluorographs. Polypeptides are those indicated on the fluorographs depicted in Figure 3, D and F.

| Polypeptides That Increase |                              |      |            |       | Polypeptides That Decrease |                              |      |            |       |
|----------------------------|------------------------------|------|------------|-------|----------------------------|------------------------------|------|------------|-------|
| PP No.                     | <i>M<sub>r</sub></i> (×1000) | pI   | CM 72      | Prato | PP No.                     | <i>M<sub>r</sub></i> (×1000) | pI   | CM 72      | Prato |
|                            |                              |      | <i>dpm</i> |       |                            |                              |      | <i>dpm</i> |       |
| 1                          | 103.7                        | 5.71 | 16         | 105   | 7                          | 68.1                         | 5.39 | 103        | 43    |
| 2                          | 98.1                         | 6.06 | 14         | 135   | 8                          | 76.7                         | 5.53 | 50         | 21    |
| 5                          | 99.2                         | 6.37 | 14         | 114   | 17                         | 53.8                         | 5.99 | 124        | 60    |
| 16                         | 60.0                         | 5.60 | 175        | 276   | 19                         | 50.1                         | 6.32 | 99         | 61    |
| 18                         | 55.0                         | 6.36 | 48         | 95    | 23                         | 43.8                         | 5.59 | 113        | 71    |
| 27                         | 36                           | 5.58 | 29         | 110   | 24                         | 45.9                         | 5.67 | 194        | 69    |
| 29                         | 41.3                         | 5.71 | 173        | 424   | 25                         | 48.0                         | 5.75 | 193        | 93    |
| 33                         | 40.0                         | 6.40 | 158        | 215   | 26                         | 45.3                         | 5.75 | 172        | 52    |
| 34                         | 42.9                         | 6.78 | 80         | 157   | 30                         | 41.3                         | 5.77 | 153        | 92    |
| 35                         | 42.0                         | 6.86 | 137        | 248   | 31                         | 42.8                         | 5.90 | 141        | 67    |
| 40                         | 36.2                         | 6.38 | 56         | 178   | 32                         | 41.4                         | 6.06 | 249        | 139   |
| 42                         | 36.9                         | 6.51 | 3          | 135   | 41                         | 37.0                         | 6.42 | 207        | 73    |
| 47                         | 30.0                         | 5.32 | 77         | 131   | 53                         | 30.9                         | 6.32 | 117        | 75    |
| 55                         | 26.8                         | 6.86 | 15         | 82    | 56                         | 30.5                         | 6.83 | 172        | 69    |
|                            |                              |      |            |       | 61                         | 18.0                         | 5.41 | 270        | 102   |
|                            |                              |      |            |       | 64                         | 17.8                         | 7.34 | 183        | 74    |
|                            |                              |      |            |       | 66                         | 70.0                         | 6.37 | 148        | 32    |

bridization experiments should be compared with results of nuclear runoff experiments to determine transcriptional rates of synthesis for those mRNAs whose levels are altered by salt. The data from these experiments will provide a better understanding of the changes caused by salt at the mRNA level *in vivo* and indicate whether or not transcription, translation, or both are affected by salt.

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