Development 136, 3543-3548 (2009) doi:10.1242/dev.036715

Coordinated activation of the secretory pathway during notochord formation in the *Xenopus* embryo

Kosuke Tanegashima*, Hui Zhao†, Martha L. Rebbert and Igor B. Dawid‡

We compared the transcriptome in the developing notochord of *Xenopus laevis* embryos with that of other embryonic regions. A coordinated and intense activation of a large set of secretory pathway genes was observed in the notochord, but not in notochord precursors in the axial mesoderm at early gastrula stage. The genes encoding Xbp1 and Creb3l2 were also activated in the notochord. These two transcription factors are implicated in the activation of secretory pathway genes during the unfolded protein response, where cells react to the stress of a build-up of unfolded proteins in their endoplasmic reticulum. Xbp1 and Creb3l2 are differentially expressed but not differentially activated in the notochord. Reduction of expression of Xbp1 or Creb3l2 by injection of antisense morpholinos led to strong deficits in notochord but not somitic muscle development. In addition, the expression of some, but not all, genes encoding secretory proteins was inhibited by injection of *xbp1* morpholinos. Furthermore, expression of activated forms of Xbp1 or Creb3l2 in animal explants could activate a similar subset of secretory pathway genes. We conclude that coordinated activation of a battery of secretory pathway genes mediated by Xbp1 and Creb/ATF factors is a characteristic and necessary feature of notochord formation.

KEY WORDS: Notochord, Protein trafficking, Protein secretion, Unfolded protein response, DNA microarray, *Xenopus*

INTRODUCTION

The notochord gives the phylum chordates its name and is a defining feature of the group of animals that includes humans. The notochord is of interest as one of the earliest organs to differentiate, because its progenitors derive from the Spemann-Mangold organizer, and it serves as a source of signals that affect the development of neighboring organs. In the embryo, the most obvious role of the notochord is to provide structural support, giving strength to the longest axis of the larva (Jiang and Smith, 2007; Scott and Stemple, 2005; Stemple, 2005). The notochord derives its stiffness from the sheath that surrounds the organ, enhanced by pressure exerted by the expanding vacuoles of notochordal cells (Adams et al., 1990; Malacinski and Youn, 1982). Formation of the sheath and vacuoles require protein export, suggesting that notochord development depends on a highly active secretory system. Important insights into notochord formation were derived from mutations in zebrafish (Stemple et al., 1996). Six genes that are important for notochord differentiation have been analyzed, three that encode components of the coatomer complex and three encoding laminins (Coutinho et al., 2004; Parsons et al., 2002; Pollard et al., 2006). An additional notochord mutant affecting collagen maturation has been reported (Gansner and Gitlin, 2008). Thus, components of the secretory machinery and secreted proteins are important for notochord formation.

We analyzed the notochord transcriptome by dissecting notochord and other regions from *Xenopus laevis* embryos and comparing their RNA populations. We find a dramatic enrichment of secretory pathway genes in the notochord RNA. The coordinated activation of a large battery of secretory pathway genes occurs in cells subjected to stress when an excess of unfolded proteins arises in the ER, leading to the unfolded protein response (UPR) (Ron and Walter, 2007; Schroder and Kaufman, 2005). This response is orchestrated by several sensing mechanisms and finally transmitted to the nucleus in the form of activated transcription factors Xbp1 and members of the ATF/Creb family (Calfon et al., 2002; Haze et al., 1999; Kondo et al., 2005; Kondo et al., 2007; Yoshida et al., 2001). Xbp1 has previously been identified in *Xenopus* embryos and shown to have a role in regulating BMP signaling, in mesoderm formation, and in the response of the embryo to ER stress (Cao et al., 2006; Yuan et al., 2007; Yuan et al., 2008; Zhao et al., 2003). We find that *xbp1* and at least one Creb family gene, *creb3l2*, are preferentially expressed in the notochord and are required for notochord differentiation. These results indicate that the coordinated expression of a large battery of secretory pathway genes in a mechanism related, but not identical, to the UPR is a key step in the formation of the notochord.

MATERIALS AND METHODS

DNA microarray experiments

Certain aspects of the DNA microarray experiments have been reported (Tanegashima et al., 2008; Zhao et al., 2008). The methods used and complete data sets are available under accession numbers E-MEXP-717 (ArrayExpress http://www.ebi.ac.uk/microarray-as/ae/), GSM227883, GSM227884, GSM227963 and GSM227964 (GEO http://www.ncbi.nlm.nih.gov/geo/). Human homologs of genes enriched in the notochord were subjected to gene ontology (GO) analysis using DAVID Bioinformatics Resources 2007 (Dennis et al., 2003).

Other methods

In situ hybridization was carried out as described (Harland, 1991; Tanegashima et al., 2008; Zhao et al., 2008). Antibodies against MZ15 (Smith and Watt, 1985) and 12-101 (Kintner and Brockes, 1984) were used. *xbp1* and *creb3l2* RNAs were injected into the animal region at levels of 50 pg per embryo, and animal caps were dissected at stage 8.5-9 and harvested after 4-5 hours. RNA was extracted using Stat-60 (TEL-TEST), purified using the RNeasy system (Qiagen) and cDNA was synthesized using SuperScript III (Invitrogen). Real-time PCR was carried out using reagents

Laboratory of Molecular Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA.

*Present address: Tokyo Metropolitan Organization for Medical Research, The Tokyo Metropolitan Institute of Medical Science, Tokyo 156-8506, Japan
†Present address: School of Biomedical Sciences, Faulty of Medicine, The Chinese University of Hong Kong, Hong Kong
‡Author for correspondence (idawid@nih.gov)

Accepted 24 August 2009

from Roche, following the manufacturer's instructions. Each sample was assayed in triplicate, and error bars in Fig. 4 represent the s.e.m. associated with these assays. At least two experiments were carried out for each condition, using independent sets of animal caps dissected from injected embryos. One of each set of experiments is shown in Fig. 4; in all cases the independent experiment(s) gave similar results to the one shown.

Morpholino injections

The MO sequences are: *xbp1* (s) MO, GAC ATC TGG GCC TGC TCC TGC TGC A (Yuan et al., 2008); *xbp1* (n) MO, GCC CAA CAA GAG ATC AGA CTC AGA G; *creb3l2* A MO, ATC CCC ACT CTC CAT TAT TTC CAT C; *creb3l2* B MO, ATC GCA GCT CTC CAT TAT TTC CAT G. *xbp1* (s) plus *xbp1* (n) MO, 30 ng each per embryo, were injected together at the two-cell stage; likewise, a mix of 30 ng each *creb3l2* A and *creb3l2* B MOs was injected. For coinjection of *xbp1* and *creb3l2* MOs, we used 15 ng of each MO, giving a total of 60 ng per embryo.

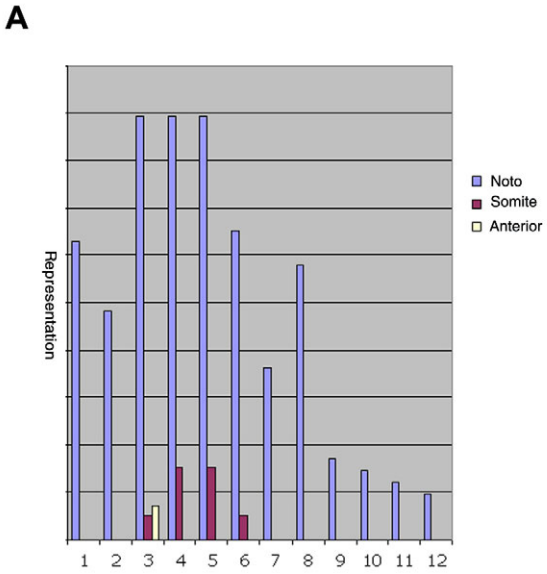
RESULTS AND DISCUSSION

Genes encoding secretory pathway proteins are preferentially expressed in the notochord

Using RNA extracted from micro-dissected regions of *Xenopus* embryos we carried out DNA microarray experiments to analyze the RNA populations at gastrula and neurula stages. We focus here on genes preferentially expressed in different regions of the dorsal mesoderm in late gastrula to early neurula embryos. The dorsal mesoderm was first subdivided into anterior (prechordal) and posterior regions, and the latter was further dissected into notochord and somites. Genes expressed preferentially in each of these regions, compared with whole embryos and with each other, were identified (Table 1). Duplicate probe sets were eliminated, and the selected genes were converted to human homologs because of better annotation (see Tables S1, S2 and S3 in the supplementary material). These tables show that multiple examples of known markers for each region were identified, supporting the validity of the selection.

Gene ontology (GO) analysis of the notochord transcriptome revealed an unusual over-representation of GO terms in one pathway. All 12 most highly represented GO terms identified components of the protein secretory pathway, at highly significant levels (Fig. 1A). These GO terms are overlapping, so that individual genes are included in multiple terms. Thus the developing notochord has a very high requirement for secretory pathway proteins (see also Introduction).

The patterns of abundance of notochord-enriched genes in different regions of the embryo at two stages of development are presented in Fig. 1B. Secretory pathway transcripts are enriched in



	Term	Count	PValue
1	secretory pathway	26	2.30E-20
2	Golgi vesicle transport	20	4.38E-19
3	protein transport	37	1.77E-18
4	intracellular transport	37	2.07E-17
5	establishment of cellular localization	37	3.14E-17
6	intracellular protein transport	27	1.18E-15
7	ER to Golgi vesicle-mediated transport	15	6.16E-15
8	vesicle-mediated transport	24	9.61E-12
9	intra-Golgi vesicle-mediated transport	7	1.65E-08
10	retrograde vesicle-mediated transport, Golgi to ER	6	2.60E-07
11	protein targeting to ER	5	6.17E-06
12	protein secretion	4	0.00242

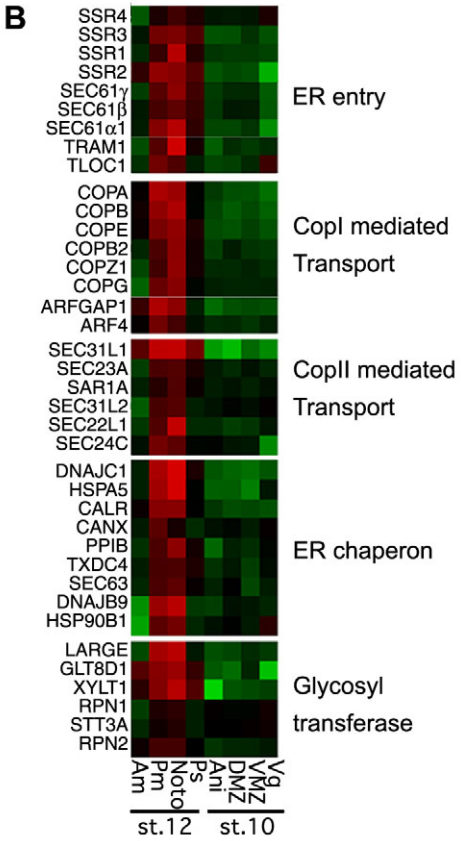


Fig. 1. High representation of secretory pathway genes in the notochord transcriptome. (**A**) The results of DNA microarray analysis on dissected *Xenopus* embryos at stage 12 (Tanegashima et al., 2008) (see also Materials and methods) were subjected to analysis of gene ontology (GO) terms. In the notochord sample, the 12 most over-represented GO terms refer to secretory pathway genes, but no such excess is seen in the somite/presomitic mesoderm or in anterior mesoderm (prechordal plate). The column labeled 'count' gives the number of genes in the notochord sample that are members of the class of the GO term listed. 'PValue' gives the probability of finding the observed GO term representation by chance. (**B**) Relative abundance of secretory pathway transcripts in dissected regions of stage 10 and stage 12 embryos, based on microarray analyses of dissected embryos (see Materials and methods) (Tanegashima et al., 2008; Zhao et al., 2008). Am, anterior mesoderm; Pm, posterior mesoderm including the notochord; noto, notochord; Ps, presomitic mesoderm; Ani, animal quadrant; DMZ, dorsal marginal zone; VMZ, ventral marginal zone; Vg, vegetal quadrant. Red represents high, and green low relative abundance. Gene names and processes in which these genes act are indicated.

Table 1. Genes preferentially expressed in dissected regions of stage 12 *Xenopus* embryos

Class	Probe sets	Unigenes	Coding sequence	Human homologs
Am>WE; Am>Pm*	167	154	119	110
Pm>Am; Pm>WE; Noto>Som†	388	355	230	219
Pm>Am; Pm>WE; Som>Noto‡	129	101	74	74

Selections for presence of probe sets and differences in abundance between samples were executed by the GCOS software in duplicate experiments. The numbers decrease because of redundancy, and because human homologs could not be identified for all *Xenopus* coding sequences.
*See Table S1 in the supplementary material.
†See Table S2 in the supplementary material.
‡See Table S3 in the supplementary material.
Am, anterior dorsal mesoderm; Pm, posterior dorsal mesoderm; WE, whole embryo; Noto, notochord; Som, presomitic mesoderm/somites.

notochord and posterior mesoderm that contains the notochord at late gastrula (stage 12), but not in the dorsal marginal zone (DMZ) at early gastrula (stage 10). Thus the secretory pathway is strongly activated in the developing notochord but not in notochord precursor cells in the axial mesoderm. This set of genes is preferentially, but not exclusively, expressed in the notochord, as illustrated by in situ hybridization (Fig. 2; see Fig. S1 in the supplementary material). Different types of secretory pathway genes are included in the notochord-enriched sample. ER entry is mediated by a protein complex containing the Sec61 heterotrimer and the associated factors TRAM and TRAPα, TRAPβ, TRAPγ and TRAPδ (SSR1, SSR2, SSR3, SSR4) (Skach, 2007). All eight components of the complex were preferentially expressed in the notochord (Fig. 1B; Fig. 2C; see Fig. S1 in the supplementary material). Tloc (Sec62) and the ER chaperone Sec63 are associated with the translocation complex (Meyer et al., 2000), and showed enhanced notochord expression (Fig. 1B, Fig. 2A; see Fig. S1 in the supplementary material). Among other genes associated with this pathway, *rrbp1*, which encodes a ribosome receptor in the rough ER (Savitz and Meyer, 1993), shows prominent expression in the notochord (Fig. 1B; Fig. 2B). Genes encoding proteins in COPI vesicle-mediated transport, a pathway that mediates retrograde trafficking of proteins from the *cis*-Golgi complex to the ER, are well represented in the notochord-enriched group. COPI proteins also mediate intra-Golgi trafficking and function in the maintenance of Golgi complex structure (Duden, 2003). This group of genes encodes coatomer proteins and Arf4, a small GTPase, and the GTPase-activating protein Arfgap1, which function together to regulate COPI trafficking (Fig. 1B; Fig. 2F,G; see Fig. S1 in the supplementary material). COPII proteins, which mediate the export of proteins from the ER, and associated factors were likewise well represented, including the Sec31 coat protein, the Sar1 GTPase, the GAP Sec23 and its adapter Sec24 (Lee and Miller, 2007), and Sec22 which interacts with Sec23/Sec24 (Fig. 1B, Fig. 2D,E; see Fig. S1 in the supplementary material). ER chaperones, such as Hsp90/Grp94, Hsp70(5a)/BiP, lectins (calnexin, calreticulin) and members of the Hsp40 family (Dnajb9, Dnajc1) (Sitia and Braakman, 2003) were highly enriched in the notochord (Fig. 1B, Fig. 2H,I,J; see Fig. S1 in the supplementary material). Finally, glycosyl transferases that mediate the maturation of secretory proteins are highly represented among notochord-enriched genes (Fig. 1B, Fig. 2K).

Many of these genes become more broadly expressed in later development. In previous work, in situ hybridization screening identified a synexpression group named ER-import that showed weak ubiquitous, moderate notochord and pronephros, and high cement gland expression (Gawantka et al., 1998). Some of the notochord-enriched secretory genes we examined overlap genes in the ER-import synexpression group. Consistently, some of the genes showed pronephros expression at tadpole stages (Fig. 2C″,E″,I″,K″).

Requirement for Xbp1 and Creb3l2 in notochord development

We found two transcription factors, Xbp1 (Zhao et al., 2003) and Creb3l2 (this study), that were enriched in notochord RNA (Fig. 3). These proteins attracted our attention because Xbp1 and Creb/ATF family factors have a key role in the UPR (Ron and Walter, 2007; Schroder and Kaufman, 2005). In the UPR, Xbp1 and Creb/ATF proteins are activated by post-transcriptional mechanisms, resulting in the coordinated transcription of a large battery of secretory pathway genes (Calfon et al., 2002; Yoshida et al., 2001; Haze et al., 1999; Kondo et al., 2005; Kondo et al., 2007). The preferential expression of Xbp1 and Creb3l2 (Cao et al., 2006; Zhao et al., 2003) (see Table S1 in the supplementary material; Fig. 3A,B) suggested that these factors might have a role in secretory pathway activation during notochord formation.

To test this possibility, two antisense morpholinos (MOs), *xbp1* (n) and *xbp1* (s), which were effective in inhibiting the nuclear splicing of *xbp1* (see Fig. S2A in the supplementary material), and the cytoplasmic splicing (Yuan et al., 2008) to active forms, were used to knock down *xbp1* expression. Also, we generated two MOs against the pseudoalleles of *creb3l2* in *X. laevis*, which are effective in blocking Creb3l2A and Creb3l2B translation (see Fig. S2B in the supplementary material). Injection of *xbp1* MO or *creb3l2* MO led to strong inhibition of notochord development, as visualized by the antibody MZ15 (Fig. 3C-F) or by in situ hybridization with *col9a1* or *sec31* (see Fig. S3 in the supplementary material). These results indicate that Xbp1 and Creb3l2 function is required for normal development of the notochord. By contrast, these MOs did not inhibit differentiation of somitic muscle as visualized using antibody 12-101 (Fig. 3G-J), supporting the specificity of the MOs. A high-level requirement for secretory proteins makes the notochord sensitive to a reduction in Xbp1 and Creb3l2, whereas somitic muscle with an apparent lower requirement for secretory proteins is resistant.

The effect of MO injection on notochord differentiation was further examined in transverse sections (see Fig. S4 in the supplementary material). At early neurula stages, there was no obvious morphological difference in the notochords of control and embryos injected with *xbp1* or *creb3l2* MOs (see Fig. S4A-C in the supplementary material). Differences were apparent by stage 37-38, when the notochord in normal embryos displayed the characteristic vacuolated appearance (see Fig. S4D in the supplementary material). Embryos injected with *xbp1* MO had a smaller notochord (see Fig. S4E in the supplementary material), whereas injection of *creb3l2* MO caused the notochord to appear much less vacuolated, suggesting a failure of differentiation (see Fig. S4F in the supplementary material). Thus the inhibition of expression of Xbp1 or Creb3l2 leads to an impairment of notochord morphology by tailbud stages of embryogenesis.

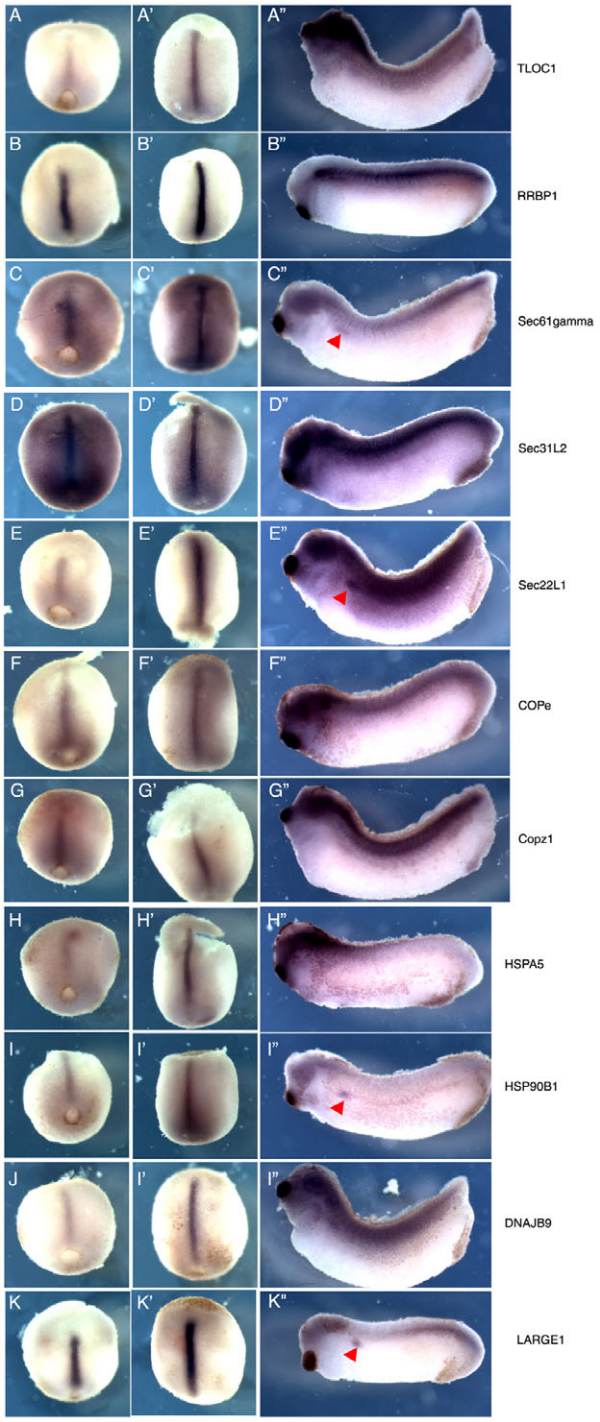


Fig. 2. In situ hybridization of selected secretory pathway genes to *Xenopus* embryos. (**A-K″**) Embryos at stage 12.5 (A-K), stages 15-17 (A′-K′) and stages 28-32 (A″-K″). All of these genes show preferential expression in the notochord, and some also show expression in the pronephros (red triangle).

Manipulation of Xbp1 and Creb3l2 protein levels affects the expression of some secretory protein genes

Xbp1 is known to have a role in *Xenopus* embryogenesis (Cao et al., 2006; Yuan et al., 2008; Zhao et al., 2003) and to be involved in the response to ER stress (Yuan et al., 2007). We tested whether

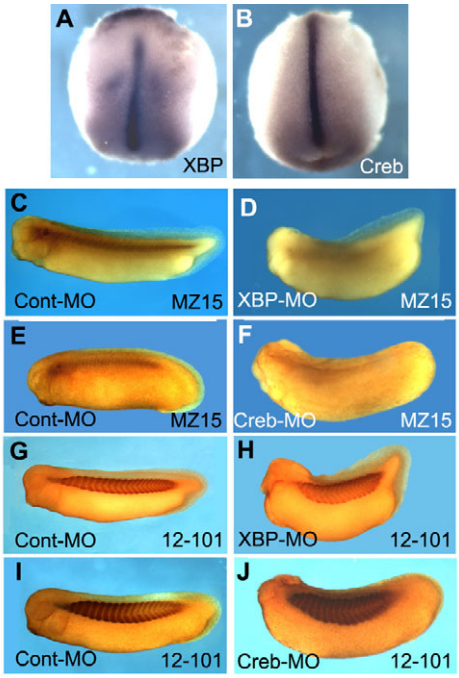


Fig. 3. XPB1 and Creb3l2 are involved in notochord formation. (**A**,**B**) In situ hybridization at stage 17 shows the preferential notochord expression of *xbp1* (A) and *creb3l2* (B). (**C-J**) Embryos were injected with control MO (C,E,G,I), a mixture of *xbp1*(n) and *xbp1*(s) MOs (D,H), or a mixture of *creb3l2* A and *creb3l2* B MOs (F,J). Embryos were stained with the notochord-specific antibody MZ15 (C-F), or the muscle-specific antibody 12-101 (G-J).

inhibition of Xbp1 or Creb3l2 affects the expression of secretory pathway genes at early and later stages of notochord differentiation. The MOs mentioned above were injected into two-cell-stage embryos, RNA was extracted at stages 12 or 18 and assayed using quantitative PCR. We chose these early stages for analysis because the obvious deterioration of notochord morphology at later stages would make RNA expression data difficult to interpret. *xbp1* MOs inhibited the expression of the genes encoding the chaperones Hsp5A/Bip, DNAJ9B and HSP90B1, whereas the *creb3l2* MOs had no clear effect (Fig. 4A,B). The expression of genes encoding three factors in the COPI pathway was not consistently affected, suggesting the possible redundant involvement of additional factors in their activation.

We also tested whether overexpression of Xbp1 or Creb3l2 could activate secretory pathway genes in the embryo. We used animal explants (caps) that do not develop into notochord on their own but can be induced to form notochord and other tissues by suitable signaling or transcription factors. We injected *xbp1*, *creb3l2* or a mixture of both RNAs into the animal region of two-cell-stage embryos, dissected animal caps at late blastula, cultured them for 4-5 hours, and measured relative levels of different transcripts by quantitative PCR. The active splice form of Xbp1 (Cao et al., 2006) and the active form of Creb3l2 [CrebΔC, carrying a deletion of the C-terminal domain (Kondo et al., 2005)] were used. As in the inhibition experiments above, genes encoding the chaperones Hsp5A/Bip, Hsp90B1 and DNAJB9 responded strongly to Xbp1 overexpression with a 7- to 17-fold increase in RNA levels (Fig. 4C). By contrast, *copa, cope* and *copz* responded quite weakly, albeit still in a significant manner. Fewer genes were activated by injection of *creb3l2*. The only gene showing a robust response was *dnaJb9*, with *hsp90b1* and *copA*

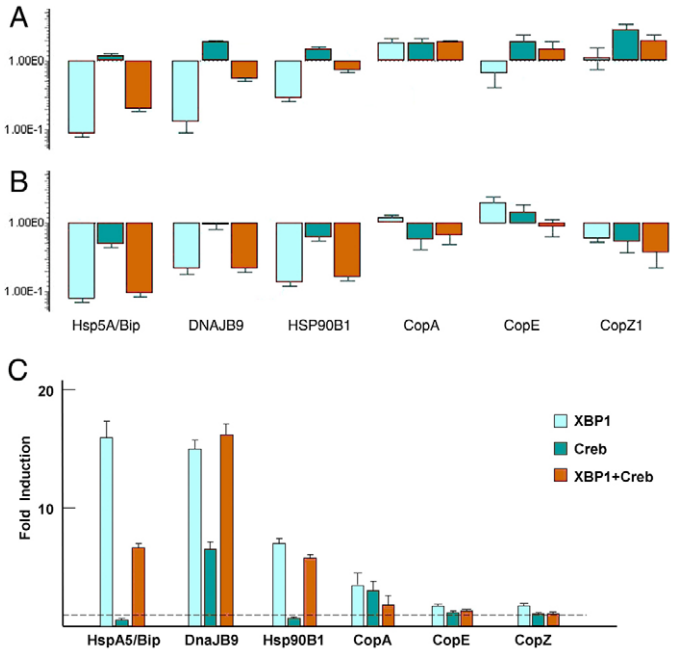


Fig. 4. Response of secretory pathway genes to manipulations of the levels of Xbp1 or Creb3l2. (**A**,**B**) Reduction of expression of some secretory pathway genes by inhibiting Xbp1 and Creb3l2 levels. MOs against *xbp1*, *creb3l2*, or both, as indicated by color coding, were injected into the equatorial region of two-cell-stage embryos, the embryos were harvested at stage 12 (A) or stage 18 (B), and RNA was extracted. The levels of various transcripts were assayed by PCR. The graphs are the direct output of the Q-PCR program, after color coding, of ratios in experimental samples compared with control-MO-injected samples. The data represent three repeat assays in one of two similar experiments. (**C**) Induction of secretory pathway genes in animal caps. RNAs were injected, and transcripts in animal caps were assayed as above, and the data represented as fold induction on a linear scale. The level of each transcript in control animal caps was set as one (dashed line). Triplicate assays of one of at least two experiments with similar results are shown.

responding weakly and the other genes not responding at all (Fig. 4C). In no case did the two transcription factors act synergistically. In addition to the genes shown in Fig. 4C, we tested *sec22*, *sec31* and *tloc*, but observed only weak responses (not shown).

The results presented above indicate that overexpression of Xbp1 or Creb3l2 alone can activate, whereas knockdown of Xbp1, but not of Creb3l2, can inhibit the expression of certain secretory protein genes in the embryo. Chaperone-encoding genes are sensitive to these manipulations whereas COPI pathway genes respond much more weakly, if at all. These data support the view that Xbp1 and Creb3l2 function in the regulation of secretory pathway genes in notochord development, and also suggest that additional factors are involved in this process.

Coordinated activation of the secretory pathway involves differential expression of Xbp1 and Creb3l2

Given the high demand for processing and trafficking of secretory proteins during notochord differentiation, the embryo faces the problem of the coordinated activation of a large set of genes. Many cells, when facing an excess of proteins entering the ER, carry out UPR, in which a rapid response is achieved by post-transcriptional activation of the relevant transcription factors (Ron and Walter, 2007; Schroder and Kaufman, 2005). The embryo appears to react somewhat differently, perhaps because the demand for secretory proteins is not a sudden physiological stress but a 'predictable' developmental process. We have asked whether post-transcriptional activation of Xbp1 or Creb3l2 is higher in the developing notochord than in ventral regions of the embryo, but could not find evidence for differential activation (see Fig. S5 in the supplementary material). Splicing of Xbp1 appeared to be the same in dorsal and ventral regions of the embryo (see Fig. S5A in the supplementary material), corresponding to the pattern previously observed in the *Xenopus* embryo (Cao et al., 2006). Likewise, injection of epitope-tagged Creb3l2 and testing for proteolytic processing in dorsal and ventral explants failed to find a difference (see Fig. S5B in the supplementary material). In contrast to this equality of activation, the levels of *xbp1* and *creb3l2* mRNAs are strongly enriched in the developing notochord (Fig. 3A,B). We conclude that the high expression of these two transcription factors is required for and greatly contributes to the dramatic coordinated activation of secretory pathway genes in the developing notochord. Thus, control of secretory gene activation in the developing notochord is primarily executed at the level of transcriptional regulation and mRNA accumulation.

Acknowledgements

We thank Lina Gugliotti for help with quantitative PCR. This work has been supported by the intramural research program of the NICHD, NIH. Deposited in PMC for release after 12 months.

Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/136/21/3543/DC1

References

Adams, D. S., Keller, R. and Koehl, M. A. (1990). The mechanics of notochord elongation, straightening and stiffening in the embryo of Xenopus laevis. *Development* **110**, 115-130.

Calfon, M., Zeng, H., Urano, F., Till, J. H., Hubbard, S. R., Harding, H. P., Clark, S. G. and Ron, D. (2002). IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* **415**, 92-96.

Cao, Y., Knochel, S., Oswald, F., Donow, C., Zhao, H. and Knochel, W. (2006). XBP1 forms a regulatory loop with BMP-4 and suppresses mesodermal and neural differentiation in Xenopus embryos. *Mech. Dev.* **123**, 84-96.

Coutinho, P., Parsons, M. J., Thomas, K. A., Hirst, E. M., Saude, L., Campos, I., Williams, P. H. and Stemple, D. L. (2004). Differential requirements for COPI transport during vertebrate early development. *Dev. Cell* **7**, 547-558.

Dennis, G., Jr, Sherman, B. T., Hosack, D. A., Yang, J., Gao, W., Lane, H. C. and Lempicki, R. A. (2003). DAVID: database for annotation, visualization, and integrated discovery. *Genome Biol.* **4**, P3.

Duden, R. (2003). ER-to-Golgi transport: COP I and COP II function (Review). *Mol. Membr. Biol.* **20**, 197-207.

Gansner, J. M. and Gitlin, J. D. (2008). Essential role for the alpha 1 chain of type VIII collagen in zebrafish notochord formation. *Dev. Dyn.* **237**, 3715-3726.

Gawantka, V., Pollet, N., Delius, H., Vingron, M., Pfister, R., Nitsch, R., Blumenstock, C. and Niehrs, C. (1998). Gene expression screening in Xenopus identifies molecular pathways, predicts gene function and provides a global view of embryonic patterning. *Mech. Dev.* **77**, 95-141.

Harland, R. M. (1991). In situ hybridization: an improved whole-mount method for Xenopus embryos. *Methods Cell Biol.* **36**, 685-695.

Haze, K., Yoshida, H., Yanagi, H., Yura, T. and Mori, K. (1999). Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. *Mol. Biol. Cell* **10**, 3787-3799.

Jiang, D. and Smith, W. C. (2007). Ascidian notochord morphogenesis. *Dev. Dyn.* **236**, 1748-1757.

Kintner, C. R. and Brockes, J. P. (1984). Monoclonal antibodies identify blastemal cells derived from dedifferentiating limb regeneration. *Nature* **308**, 67-69.

Kondo, S., Murakami, T., Tatsumi, K., Ogata, M., Kanemoto, S., Otori, K., Iseki, K., Wanaka, A. and Imaizumi, K. (2005). OASIS, a CREB/ATF-family member, modulates UPR signalling in astrocytes. *Nat. Cell Biol.* **7**, 186-194.

Kondo, S., Saito, A., Hino, S., Murakami, T., Ogata, M., Kanemoto, S., Nara, S., Yamashita, A., Yoshinaga, K., Hara, H. et al. (2007). BBF2H7, a novel transmembrane bZIP transcription factor, is a new type of endoplasmic reticulum stress transducer. *Mol. Cell. Biol.* **27**, 1716-1729.

Lee, M. C. and Miller, E. A. (2007). Molecular mechanisms of COPII vesicle formation. *Semin. Cell Dev. Biol.* **18**, 424-434.

Malacinski, G. M. and Youn, B. W. (1982). The structure of the anuran amphibian Notochord and a re-evaluation of its presumed role in early embryogenesis. *Differentiation* **21**, 13-21.
Meyer, H. A., Grau, H., Kraft, R., Kostka, S., Prehn, S., Kalies, K. U. and Hartmann, E. (2000). Mammalian Sec61 is associated with Sec62 and Sec63. *J. Biol. Chem.* **275**, 14550-14557.
Parsons, M. J., Pollard, S. M., Saude, L., Feldman, B., Coutinho, P., Hirst, E. M. and Stemple, D. L. (2002). Zebrafish mutants identify an essential role for laminins in notochord formation. *Development* **129**, 3137-3146.
Pollard, S. M., Parsons, M. J., Kamei, M., Kettleborough, R. N., Thomas, K. A., Pham, V. N., Bae, M. K., Scott, A., Weinstein, B. M. and Stemple, D. L. (2006). Essential and overlapping roles for laminin alpha chains in notochord and blood vessel formation. *Dev. Biol.* **289**, 64-76.
Ron, D. and Walter, P. (2007). Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.* **8**, 519-529.
Savitz, A. J. and Meyer, D. I. (1993). 180-kD ribosome receptor is essential for both ribosome binding and protein translocation. *J. Cell Biol.* **120**, 853-863.
Schroder, M. and Kaufman, R. J. (2005). The mammalian unfolded protein response. *Annu. Rev. Biochem.* **74**, 739-789.
Scott, A. and Stemple, D. L. (2005). Zebrafish notochordal basement membrane: signaling and structure. *Curr. Top. Dev. Biol.* **65**, 229-253.
Sitia, R. and Braakman, I. (2003). Quality control in the endoplasmic reticulum protein factory. *Nature* **426**, 891-894.
Skach, W. R. (2007). The expanding role of the ER translocon in membrane protein folding. *J. Cell Biol.* **179**, 1333-1335.
Smith, J. C. and Watt, F. M. (1985). Biochemical specificity of Xenopus notochord. *Differentiation* **29**, 109-115.
Stemple, D. L. (2005). Structure and function of the notochord: an essential organ for chordate development. *Development* **132**, 2503-2512.
Stemple, D. L., Solnica-Krezel, L., Zwartkruis, F., Neuhauss, S. C., Schier, A. F., Malicki, J., Stainier, D. Y., Abdelilah, S., Rangini, Z., Mountcastle-Shah, E. et al. (1996). Mutations affecting development of the notochord in zebrafish. *Development* **123**, 117-128.
Tanegashima, K., Zhao, H. and Dawid, I. B. (2008). WGEF activates Rho in the Wnt-PCP pathway and controls convergent extension in Xenopus gastrulation. *EMBO J.* **27**, 606-617.
Yoshida, H., Matsui, T., Yamamoto, A., Okada, T. and Mori, K. (2001). Xbp1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* **107**, 881-891.
Yuan, L., Cao, Y. and Knochel, W. (2007). Endoplasmic reticulum stress induced by tunicamycin disables germ layer formation in Xenopus laevis embryos. *Dev. Dyn.* **236**, 2844-2851.
Yuan, L., Cao, Y., Oswald, F. and Knochel, W. (2008). IRE1beta is required for mesoderm formation in Xenopus embryos. *Mech. Dev.* **125**, 207-222.
Zhao, H., Cao, Y. and Grunz, H. (2003). Xenopus X-box binding protein 1, a leucine zipper transcription factor, is involved in the BMP signaling pathway. *Dev. Biol.* **257**, 278-291.
Zhao, H., Tanegashima, K., Ro, H. and Dawid, I. B. (2008). Lrig3 regulates neural crest formation in Xenopus by modulating Fgf and Wnt signaling pathways. *Development* **135**, 1283-1293.