MODULATION OF C-FOS PROMOTER ACTIVITY BY BRIEF LOW AMPLITUDE STRAINS: A ROLE FOR ETS TRANSCRIPTION FACTORS?

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INTRODUCTION:
Osteoblastic cells respond to mechanical forces by activating signal transduction cascades and altering gene expression patterns. In this study, we examined the responses of MC3T3-E1 mouse osteoblasts to short term, low level (1000µε, 1Hz) loads applied by cyclic deformation of the growth surface. At these load levels, daily short-term loading significantly retards the ascorbate-induced differentiation of the cells as measured by alkaline phosphatase (ALP) and osteopontin expression. This effect peaked at 5 minutes of loading per day. Loads of 1 or more hours per day duration accelerated the differentiation process slightly, as measured by the same criteria. The mechanism underlying this dichotomous, cycle number dependent response of the MC3T3-E1 cells to mechanical deformation is as yet unknown. We are focusing on possible transcriptional mechanisms by using promoters that respond to these short loading bouts as probes to identify transcription factors that may be involved. One such promoter drives the expression of c-fos, and is the focus of this study.

METHODS:

• Cells & Cell culture: The mouse osteoblastic cell line MC3T3-E1 was used in these experiments. Cells were maintained in MEM containing 10% FBS. The medium was supplemented with ascorbate for the experiments. As measured by ALP activity, the ascorbate-induced differentiation of these cells was significantly delayed by 60 minutes of loading. Neither of these sites appear to be required for the response to 60 minutes of loading. The identity of the factor that binds to the ets site remains unknown at this time.

• Mechanical loading: Cells were loaded using a vacuum based cell loading device, which deforms standard 60mm polystyrene cell culture dishes. In this study, we used ±1000 µε at 1Hz for from 1 minute to 1 hour daily for up to 14 days. For the fos promoter experiments, cells were loaded for 0, 5 or 60 minutes. The principal advantages of our loading system are derived from the use of the rigid polystyrene cell culture dishes. This allows us to drive the system with high vacuum and pressure differentials, which results in rapid response times. The resulting strains at the dish surface are, however small, and within the range of strains that are predicted to occur physiologically at bone surfaces. Furthermore, vertical displacements of the dish bottom center point are less than 1 mm, which reduces fluid inertial reaction forces.

• Promoter constructs: The murine c-fos promoter constructs used in this study include 356 BP (from the start of transcription) of the wild type (wt) promoter, and two mutants at the serum response element site. The c-fos serum response element contains an ets binding site, which binds members of the ets family of transcription factors in responses to mechanical input in osteoblasts. Since ets factors frequently regulate transcription by complexing with other transcription factors, clustered ets binding sites have been implicated in responses to mechanical input in osteoblasts. We have focused initially on the Serum Response Element as a likely candidate for a mechanically influenced transcriptional response. Factors that bind to this site have been implicated in responses to mechanical input in other cell types. The c-fos promoter Serum Response Element contains both a CArG motif responsible for binding the Serum Response Factor and an ets core motif CAGGAT that can bind ets factors. Ets family transcription factors in particular have been implicated in gene regulation in response to mechanical stimulation in several systems.

The response of the c-fos promoter mutant in which the ets binding site was destroyed to 60 minutes of loading was indistinguishable from that of the wt-promoter. In contrast the ets-mutated promoter responded to a 5 minute loading with a rapid increase in activity (~150%), which peaked at 1 hour before returning to baseline at 24 hours. The activity of the CArG box mutant, which inhibits SRF binding, is inhibited by loading to a similar extent as the wt. These results suggest that an intact ets binding site, but not necessarily an intact CArG box, is required for the transcriptional inhibition of the fos promoter by 5 minutes of loading. Neither of these sites appear to be required for the response to 60 minutes of loading. The identity of the factor that binds to the ets site remains unknown at this time.

DISCUSSION:
The biological significance of the divergent responses of osteoblasts to these very brief loading bouts is not known. Both 5 and 60 minute loading regimens inhibited c-fos promoter activity. However, the inhibition by 60 minutes of loading was very transient, returning to baseline by 24 hours, while the response to 5 minutes of loading was more sustained. In addition, our results suggest that the inhibition of the c-fos promoter by 5 and 60 minute loading bouts are regulated by different mechanisms, and implicates the et protein family of transcription factors in the response to the briefier loading events. It is not known if there is a causal relationship between the responses of the c-fos promoter and the rate of differentiation of the cells. However, the differences between the responses to shorter and longer loading episodes suggest the existence of a mechanism by which the cell can integrate the number of loading events perceived in it's recent history. We postulate that such a mechanism would be a necessary part of a loading threshold-dependent bone adaptation system.

Our understanding of the mechanisms of promoter inhibition lags behind that of activation. Nevertheless, as transcriptional modulation of gene expression is the net sum of the stimulatory and inhibitory influences acting upon the basal transcriptional machinery. Thus transcriptional inhibition plays a critically important role, which must be considered if the regulation of any gene is to be completely understood. The ets transcription factors belong to a ~30 member family which contain a conserved 85 BP DNA binding domain. Their target sequence is the ets binding site, and they have been implicated in the control of proliferation, differentiation, transformation & apoptosis in various cell systems. In addition to interacting with their own DNA binding site, they frequently regulate transcription by complexing with other transcription factors. They contain specific domains for interaction with HLH, bHLH, and Paired type transcription factors. Most are transcriptional activators, however they can act as transcriptional repressors by displacing activators masking/blocking activation domain of activators affecting chromatin structure/accessibility at gene of interest or, in the case of Yan, Ert, Net/Sap2/Erp, and Tel/ETV6, interfering with basal transcriptional machinery. The molecular identification of the factor binding to the ets site in mechanically stimulated osteoblasts, and understanding its interaction with the adjacent SRF and the mechanism by which the inhibition occurs, are a high priority.

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