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Synaptic activity–responsive element (SARE)

A unique genomic structure with an unusual sensitivity to neuronal activity

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Formation of a new memory requires plasticity at the synaptic level. However, it has also been shown that the consolidation and the maintenance of such a new memory involve processes that necessitate active mRNA at the nucleus of the cell. How can robust changes in synaptic efficacy specifically drive new transcription and translation of new gene transcripts, and thus transform an otherwise transient plasticity into a long-lasting and stable one? In this article, we highlight the conceptual advance that was gained by the discovery of a potent Synaptic Activity–Responsive Element (SARE) found ~7 kb upstream of the transcription initiation site of the neuronal immediate early gene Arc. The unique genomic structure of SARE, which contained adjacent and cooperative binding sites for three major activity-dependent transcription factors within a 100-bp locus, was associated with an unusual responsiveness to neuronal stimuli. Taken together, these findings shed light on a new class of transcriptional sensor with enhanced sensitivity to synaptic activity.

Key words: CREB, Arc, calcium, MEF2, SRF, CaMK, synaptic activity, plasticity

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Strengthening and weakening of synaptic connectivity have been thought to underlie the plastic changes that occur within neuronal circuits, when an animal has once experienced a robust environmental change such that its context and content have to be recalled over time for this animal to survive.[1] Protein synthesis inhibitors have been shown to block such storage of new information. One influential hypothesis in the field of learning and memory (termed "the synaptic tagging and capture hypothesis")[2] postulated that formation of a robust memory may trigger plasticity at stimulated synapses, while also may strongly induce expression of specific sets of genes in the nucleus. The subsequent translation and the trafficking of these newly transcribed genes into the plastic "tagged" synapses appeared to provide an attractive mechanism that could successfully account for the segregation of stably plastic synapses (in which "tags" were able to functionally "capture" new gene products) from weakly plastic ones (which could not capture any).

What could be the transcriptional regulators that are activated in conjunction with synaptic plasticity? Are there many of them? One of the obvious candidates is the cyclic AMP-responsive element binding protein (CREB), a transcription factor that has been shown to be involved in long-term memory formation, consolidation and reconsolidation.[3-8] Adult mice with disrupted CREB function in the brain exhibited a profound and specific impairment in long-term memory while the short-term memory remained unaltered.[3,5,6] Consistent with a potential role of CREB, several intracellular signaling pathways that are stimulated by synaptic activity, such as CaMKK-CaMKIV cascade, cAMP/PKA stimulation or Ras/Raf/MEK/ERK cascade have also been shown to elevate levels of CREB phosphorylation status.[9-13]

However, more than 5% of mammalian genes appear to be potentially

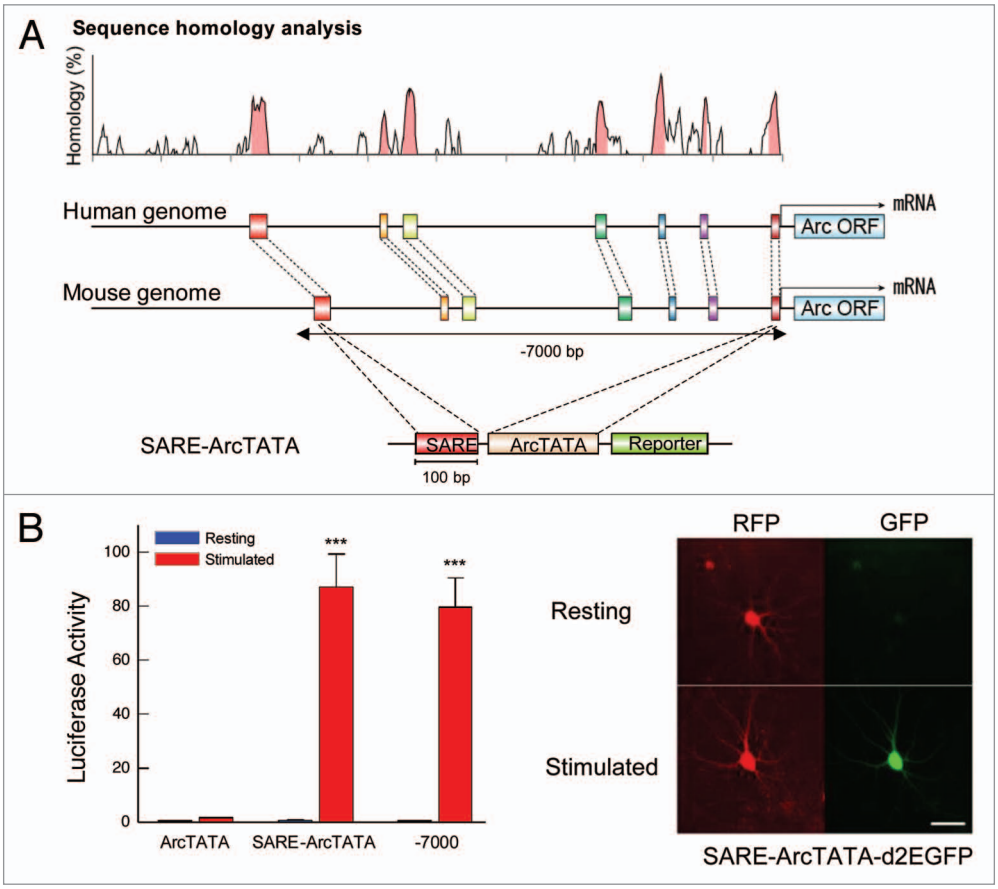

Figure 1. Synaptic Activity–Responsive Element (SARE) possesses a strong enhancer activity that is uniquely sensitive in response to synaptic stimulation. (A) SARE locates in an evolutionarily conserved genomic region in the *Arc* promoter. The SARE transcriptional activity was investigated with a construct in which SARE was fused directly upstream of a short TATA-containing sequences of the Arc promoter (SARE-ArcTATA). (B) SARE replicates the activation ability of the Arc7000 full promoter. Left, Summary of luciferase assays. Cultured cortical neurons electroporated with luciferase reporter constructs were synaptically activated and luciferase induction-folds were measured. Right, GFP reporter assays. GFP reporter expression via SARE activation was detected in individual neurons. RFP served as a transfection marker. Modified from Kawashima et al.[30] Scale bar, 50 μm.

regulated by CREB-dependent transcription, as determined by genome-wide analyses of CREB-bound promoters.[14,15] How can we then pinpoint the major CREB target genes that are involved in long-term memory maintenance? For example, c-*fos* or brain-derived neurotrophic factor (BDNF) genes possess well-known consensus CRE elements in their proximal promoter regions.[16-18] In sharp contrast, although many recent studies had highlighted the critical importance of Activity-regulated cytoskeleton-associated protein (Arc) gene,[19-25] both as an accurate and sensitive marker for enhanced cognitive activity,[26,27] and as a memory-forming gene,[23,28] no functional CRE site was reported.[29] Was Arc induced by synaptic activity by a mechanism that did not require CREB? Or were there hidden CRE sites that needed to be revealed?

Kawashima et al.[30] employed an ameliorated promoter assay system in cortical neuronal cultures that was optimized for synaptic stimulation and gene transfer of large plasmids. Through careful promoter analyses, the critical responsible element was pinned down to about 100-bp, which was named Synaptic Activity-Responsive Element (SARE). This element, when placed in isolation next to a minimal promoter region, could still trigger an extremely high level of gene induction upon receipt of synaptic activity, to an extent comparable to the full-length ~7-kb promoter (**Fig. 1**). By scrutinizing the SARE sequence, and through a combination of electrophoretic mobility shift and chromatin immunoprecipitation assays, it became evident that SARE had a unique structure consisting of a half CRE site that was juxtaposed to a MEF2 site and an SRF/TCF site. Point mutations in either one of these sites potently attenuated the synaptic activity-dependency, indicating that the CREB-, MEF2- and SRF-binding sites within SARE needed to be co-occupied in order to reach full potency. Pharmacological experiments determined the requirement for both CaMK- and MAPK-dependent pathways in this activation process (**Fig. 2**).

This study has shed light on the new exciting possibility that the co-occupancy of 3 major activity-dependent transcription factor sites in close proximity within SARE is not only necessary but also sufficient to trigger an unusually large synaptic activity-induced transcriptional response. Future studies are clearly needed to elucidate how neuronal activity can regulate the occupancy of individual sites, and determine the sustainability of this

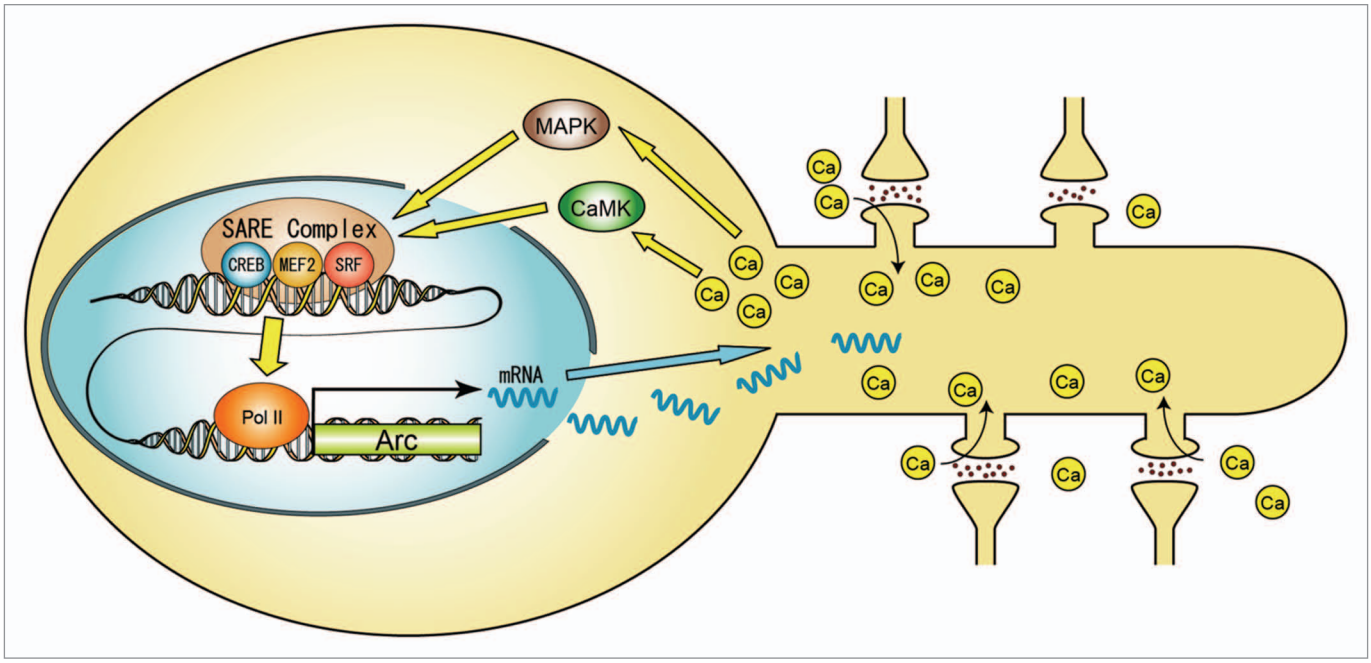

Figure 2. A model of activity-dependent regulation of the Arc gene. Ca^{2+} entry through NMDA-receptors activates CaMK- and MAPK-pathways, which in turn activate 3 major acivitity-responsive transcription factors, CREB, MEF2 and SRF at the SARE site. A putative SARE-protein complex interacts with the transcriptional machinery to promote Arc gene epxression.

putative transcriptional complex. Finally, the potential role of Arc as a gene product captured and working at "tagged" synapses still needs intense investigation. Further deciphering of the signaling from synapse to the nucleus and back to the original stimulated synapses will hopefully pave the way for better understanding of cognitive disorders including mental retardation and memory deficits.

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