However, the effective role of DA, and of its receptors, in the modulation of GI functions is far from being clear. Thus, the aim of this study was to explore the role of DA in the GI tract, using as model the mouse distal colon, analyzing, *in vitro*, spontaneous and neurally-evoked mechanical activity of the circular muscle. DA caused a direct inhibitory effect on the colonic spontaneous contractions, antagonized by SCH-23390, D1 receptor antagonist, and by domperidone, D2 receptor antagonist. In addition, DA induced a significant decrease in the amplitude of the neurally-evoked cholinergic contractions, affected by SCH-23390 and by L-NAME, nitric oxide (NO) synthase inhibitor, but not by domperidone. SCH-23390 *per se* increased the amplitude of both spontaneous and neurally-evoked cholinergic contractions. In conclusion, in mouse distal colon, dopamine is a negative modulator of GI motility *via* activation of D1 and D2 receptors. Both receptors are available for pharmacological recruitment, even if only D1-like receptors appear to be preferentially stimulated by endogenous DA. D1 receptors slow down the mouse colonic motility, reducing acetylcholine release from ENS *via* a NO-dependent pathway.

The Sea Urchin Sns5 Chromatin Insulator Settles A Gene Therapy Vector Into An Independent Domain Of Expression In The Vertebrate Genome

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One critical aspects of introducing a transgene into the eukaryotic genome is the great variability of gene expression due to position effects(1). Chromatin-dependent repressive states could be overcome by incorporation in the transgene of chromatin insulators, functioning to establish domains of expression. We have previously demonstrated that the sea urchin sns5 DNA element has typical features of an insulator: by acting as enhancer blocker, it shields promoters from neighboring regulatory elements, and by acting as barrier it buffers a transgene from the propagation of condensed chromatin(2-4).
We have investigated the use of sns5 in the field of gene therapy. Our preliminary studies shown that the inclusion of sns5 in γ-retroviral vectors allows position-independent expression in erythroid cells. Moreover, transcription factors and histone modifications mark the sns5 chromatin at the integration site(5), suggesting that sns5 displays mechanisms of action common to other well characterized insulators.
Here we show that sns5 increases the likelihood and the expression of a β-globin/lentiviral vector integrated as a single copy in both murine cell clones and in a mouse model of β-thalassemia.
It has been proposed that two copies of insulators may direct the formation of a chromatin loop by interaction among protein complexes assembled on their sequences(6).Intriguingly, by using the 3C technology, we found that sns5-flanked vectors integrated at a single copy in the genome are specifically organized into an independent chromatin structure.
Our findings highlight that sns5 could be a promising tool for improving the performance of vectors in the field of gene therapy.

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