

look for a possible impact of the insertion site neighbourhood upon the transgene expression and phenotypic effects.

By linkage mapping, we were able to confirm placement of the transgene to the proximal segment of RNO11, the critical interval being 4 cM between *D11Rat20* and *D11Rat21* (Fig. 3a, 3b). This result confirmed the FISH analysis and represents the first step towards detailed mapping and identification of the molecular basis of increased renal expression of the transgene. Markers *D11Rat20* and *D11Rat21* are placed in the rat radiation hybrid (RH) map at positions 617.4 and 568.4, respectively, with logarithm of odds (LOD) > 3.0. The distance between them is 49 cR<sub>3000</sub> (<http://rgd.mcw.edu/>) and if an average of 12.5 cR<sub>3000</sub>/1 cM is taken (Watanabe et al., 1999), our linkage results are in good agreement with the RH map. Moreover, the map bin defined by the RH positions allowed us to identify RH mapped ESTs and genes that could be considered to be candidate sequences possibly influencing the transgenic *Cd36* expression. The interval contains eight Unigene clusters and genes according to [http://rgd.mcw.edu/tools/vcmap/vcmap.cgi?MapName=Rat+VirtualMap+5.0\\_HS&Chr=11&FirstPos=568.4&SecondPos=617.4](http://rgd.mcw.edu/tools/vcmap/vcmap.cgi?MapName=Rat+VirtualMap+5.0_HS&Chr=11&FirstPos=568.4&SecondPos=617.4)). Several of the candidates, situated between *D10Rat20* and *D10Rat21*, are known to be expressed in kidney: *App* (amyloid β(A4) precursor protein, I11926), Rn.5790 (*Atp5j* – ATP synthase, H<sup>+</sup> transporting, mitochondrial F0 complex, subunit F6), Rn.40214 (ESTs, homologous to Mm.31546 ESTs) and Rn.16839 (ESTs). However, the expression of these genes and ESTs is not restricted to kidney (Table 1).

Generally, the low copy number is probably associated with increased transgene expression in the SHR-TG19 compared to the SHR-TG10 line. However, it is possible that the strong transgene expression in kidney might be influenced by the genomic context of *Cd36* insertion in proximal RNO11. In turn, the increased renal expression of *Cd36* might determine the phenotypic differences between TG19 and TG10 transgenic lines. Several genes in the critical region are expressed in kidney; therefore, some regulatory sequences driving renal expression should exist there. Consequently, one might speculate that if the *Cd36* transgene were inserted in the vicinity of the above-mentioned regulatory sequences, they might enhance the expression of the *Cd36* transgene. Although the mechanism of the strong expression in kidney could be hypothesized, the exact explanation will need cloning and precise molecular characterization of the insertion site. The most straightforward approach to define the flanking DNA sequences seems to be sequence analysis of inverse PCR products (e.g. Williams et al., 2002).

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