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A high-resolution map around the locus *Om* on mouse Chromosome 11

P.A. Baldacci, M. Cohen-Tannoudji, C. Kress, S. Pournin, C. Babinet

Unité de Biologie du Développement, U.R.A.C.N.R.S. 1960, Institut Pasteur, 25 Rue du Dr. Roux, Paris 75724 Cedex 15, France

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Abstract. The locus *Om* (ovum mutant) identified in the mouse strain DDK affects the viability of (DDK × non-DDK)F_1 preimplantation embryos. We previously located this locus on Chromosome (Chr) 11 close to *Scya2* (Baldacci et al. Mamm. Genome 2, 100–105, 1992). Here we report a high-resolution map of the region around *Om* based on a large number of backcross individuals. The same region has been analyzed on the EUCIB backcross, and the two maps have been compared. The results define the proximal and distal boundaries for the *Om* mutation as *Scya2* and *D11Mit36* respectively. The distance between these two markers is about 2 cM. These data should facilitate the positional cloning and molecular characterization of *Om*.

Introduction

The DDK syndrome was first described by Wakasugi and associates (1967, 1973). When DDK females are mated to non-DDK males, the F_1 embryos die around the morula to blastocyst stage. The proportion of embryos that fail to develop depends on the non-DDK strain. The reciprocal crosses, non-DDK females × DDK males, are fertile. Wakasugi proposed that a locus, *Om,* is responsible for the presence of a factor O in the ova and a product S in the sperm (Wakasugi 1974). The alleles *O* and *S* would be present in the majority of mouse strains, and the alleles *o* and *s* in DDK. The model proposes that the combination *o* and *S* is lethal for the embryo, whereas all other combinations are viable. We have shown that this lethality is due to an incompatibility between a DDK maternally encoded cytoplasmic product and the non-DDK paternal genome (Renard and Babinet 1986; Babinet et al. 1990). More recently the cytoplasmic factor has been shown to be present in DDK oocytes as an RNA (Renard et al. 1994). However, nothing is known about the molecular mechanisms responsible for this incompatibility. An initial study (Baldacci et al. 1992) on recombinant inbred strains between DDK and BALB/c together with an analysis of a backcross between BALB/c females and (BALB/c × DDK)F_1 males showed that *Om* was located on Chr 11 near *Scya2,* formerly *Sigje* (Loosie et al. 1994). An independent study gave a similar location (Sapienza et al. 1992). The genetic interval defined by these two studies was not sufficiently precise to consider undertaking a positional cloning of *Om.* We have, therefore, analyzed a large number of backcross individuals segregating *Om* alleles to define a small interval of recombination that would be compatible with cloning in yeast artificial chromosomes.

Materials and methods

Mouse strains and DNA samples. BALB/c and DDK mice are from our breeding colonies at the Pasteur Institute. Two series of reciprocal recombinant inbred strains (CXDD and DDXC) were established and have been previously described (Baldacci et al. 1992). The first generation backcross (BC1) between BALB/c females and a (BALB/c × DDK)F_1 male has also been described (Baldacci et al. 1992). BC1 males that were heterozygous at the *Om* locus were mated to BALB/c females to obtain BC2 progeny. This mating protocol was continued until the BC6 generation.

The EUCIB backcross has been described previously (Breen et al. 1994). The specimens in the LS series ((SPR × C57Bl/6)F_1 × SPR) which are recombinants between the markers *Csfgm* and *D11Mit10* were analyzed.

Analysis of Om. The recombinant BC males were typed for the *Om* allele in an in vitro assay as previously described (Baldacci et al. 1992). Briefly, DDK females were superovulated and mated to BC males. The embryos were isolated after 48 h and cultured in vitro. The number of morulas that developed into blastocysts was recorded. The male was typed as BALB/c when 10% or less of the embryos developed, and as F_1 when about 50% of the morulas gave rise to blastocysts.

Analysis of microsatellite markers. Polymerase chain reactions (PCR) were performed on tail DNAs as described by Love and associates (1990). PCR products were run on 4% agarose gels. The oligonucleotide primers were obtained from Research Genetics (Huntsville, Ala.) or synthesized by Genset (Paris). The primers used were from the D11Mit series (Dietrich et al. 1994), as described by Aitman and colleagues (1991) for *Scya2* (formerly *Sigje;* Loosie et al. 1994), and as described by Hearne and coworkers (1991) for *Mpo* and *Csfgm.* Primers for *Scya1* (formerly *Tca-3;* Loosie et al. 1994) were forward GGGCTGGAGGAAGGTTATGG and reverse GGAGGATGAAGAGGAGGAGG. *Nos1* was renamed *Nos2* to conform to human nomenclature (Jenkins et al. 1994).

The distances in the RI strains were calculated as described by Silver (1985). The distances in the backcrosses were calculated as the frequency of recombinants, with the 95% confidence interval of the distance given in square brackets.

Results

Segregation of markers in recombinant inbred strains. Twenty-four RI strains derived from the parental strains BALB/c and DDK were analyzed. The genotypes are summarized in Fig. 1. Eight strains recombined between the most proximal marker *Csfgm* and the group of microsatellites *D11Mit34, 94, 96, 118,* and *144,* indicating that they are loosely linked. However, only one recombinant inbred strain was found to have recombined between the markers *D11Mit34, 94, 96, 118, 144* and *D11Mit33, 35, 36, 93, 119, 195, 211, Scya1, 2, Om.* Another strain had recombined between this latter group and *D11Mit38, 39, Mpo.* The estimated genetic distances are *D11Mit34, 94, 96, 118, 144*–1.11cM (0.03–7.73cM)–*D11Mit 33, 35, 36, 93, 119, 195, 211, Scya1, 2, Om*–1.11cM (0.03–7.73cM)–*D11Mit 38, 39, Mpo.* This order and the distances are consistent with the published data using other RI strains (Loosie et al. 1994; Blackburn et al. 1995).

Segregation of markers in backcross progeny. In total, 405 BC individuals have been analyzed. The genotype distribution is sum-

Correspondence to: P. Baldacci

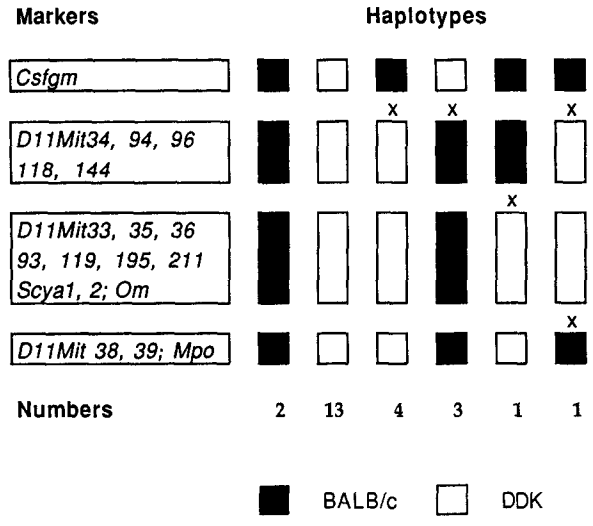

Fig. 1. Distribution of haplotypes in 24 recombinant inbred strains established from DDK and BALB/c. The recombination events are shown as x.

marized in Fig. 2. The order of markers and the genetic distances at the 95% confidence interval, calculated from these data, are: *D11Mit34*–0.6cM [0.0–3.4cM]–*D11Mit94, 96, 144*–1.67cM [0.5–4.2cM]–*D11Mit118*–0.85cM [0.2–2.5cM]–*D11Mit33, 93, Scya2*–0.28cM [0.0–1.6cM]–*Scya1, Om*–1.55cM [0.6–3.3cM]–*D11Mit35, 36*–0.26cM [0.0–1.4cM]–*D11Mit38, 39, Mpo*. We previously found that *Om* was linked to *Scya2*. These new data define *Scya2* as the closest proximal marker to *Om*, and *D11Mit 36* as the closest distal marker. It should be noted that no recombinant was found for 405 individuals analyzed between *Om* and *Scya1*. *Scya1* is, therefore, the closest known marker to *Om* at a maximum distance of 0.74 cM (95% confidence level).

Segregation of markers in the EUCIB backcross. The LS series ((SPR × C57Bl/6)F_1 × SPR) contains 427 samples, of which 207 are recombinants on Chr 11 between the markers *Csfgm* and *D11Mit10*. DNAs from 201 recombinant samples were analyzed with microsatellites in the region around *Om*. Although this backcross does not provide information on the position of *Om*, it was analyzed to provide an independent high-resolution map around the markers *Scya1* and *2*, which could be compared to the BC map. The genotypes are summarized in Fig. 3. The calculated distances between the markers are: *Csfgm*–8.4cM [6.0–11.5cM]–*D11Mit30*–0.23cM [0.0–1.3cM]–*Atp1b2, D11Mit31*–1.4cM [0.5–3.0cM]–*Nos2, D11Mit94, 96, 144*–0.23cM [0.0–1.3cM]–*D11Mit92, 95*–0.47cM [0.0–1.7cM]–*D11Mit33, 247*–0.47cM [0.0–1.7cM]–*D11Mit37, Scya1, Scya2*–0.47cM [0.0–1.7cM]–*D11Mit36*–36.5cM [32–41.3cM]–*D11Mit10*.

A comparison of the two maps obtained from these backcrosses shows that the order of the markers *D11Mit94, 96, 144, 33, 37, Scya1, Scya2* and *D11Mit36* is respected. This indicates that there are no major rearrangements in the DDK mice in this region. Furthermore, there is no significant difference in the distances obtained in these two crosses between the markers *D11Mit94, 33,* and *36*. These data have been compared with the several published maps of Chr 11 (Loosie et al. 1994; Wakana et al. 1994; Blackburn et al. 1995) and with the MIT and EUCIB maps in the data banks. It is noteworthy that the analysis of this large number of backcross individuals gives a better precision for the localization of the *Scya* family of genes than with the consensus map, *Scya2* being proximal to *Scya1*, itself proximal to *Scya3* (*D11Mit35*). Concerning the markers on the MIT and EUCIB map, it is interesting to note that we have also separated the markers *D11Mit33, 94, 96, 118,* and *144* into three recombination groups (*D11Mit94, 96, 144*), (*D11Mit118*), and (*D11Mit33*), and we have been able to separate *D11Mit37* from *D11Mit35* and *D11Mit36*. Unfortunately, the relative positions of *D11Mit35* and *36* could not be determined, since they cosegregated in our backcross and *D11Mit35* was not polymorphic in our assay on the EUCIB backcross.

Discussion

The data reported here provide a detailed map of Chr 11 around the mutation *Om*. We have been able to separate and define more precisely a number of markers on the consensus maps and the MIT and EUCIB maps. This analysis has allowed us to define two boundary markers, *Scya2* and *D11Mit 36*, around *Om* that are

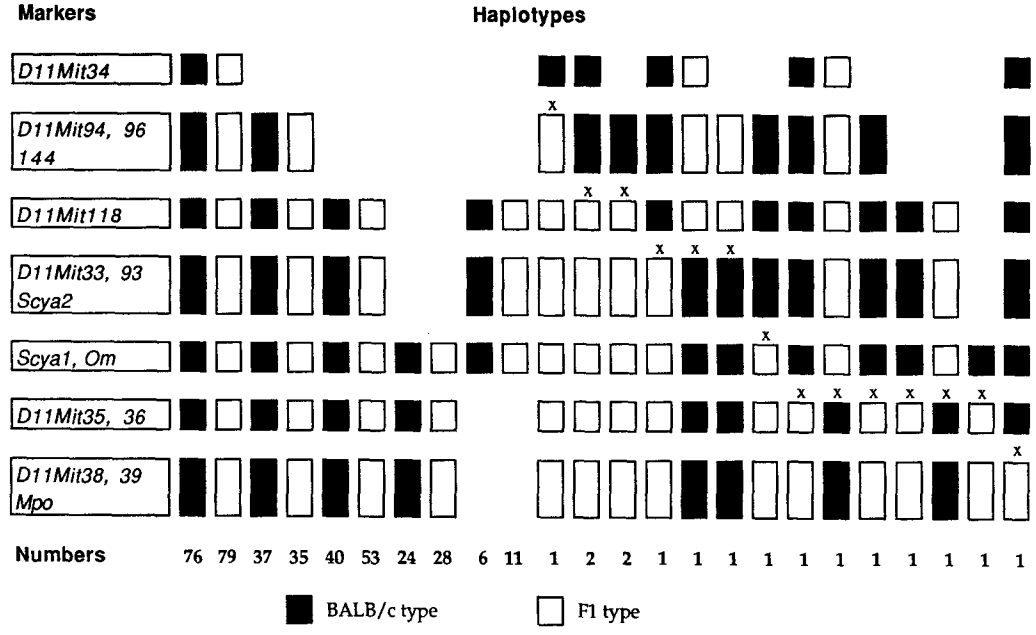

Fig. 2. Distribution of haplotypes in 405 samples from the offspring of the backcrosses BALB/c × (BALB/c × DDK)F_1. The absence of haplotypes in some columns is owing to specimens that were obtained from a recombinant parent and therefore noninformative for certain markers. Recombination events are indicated by x.

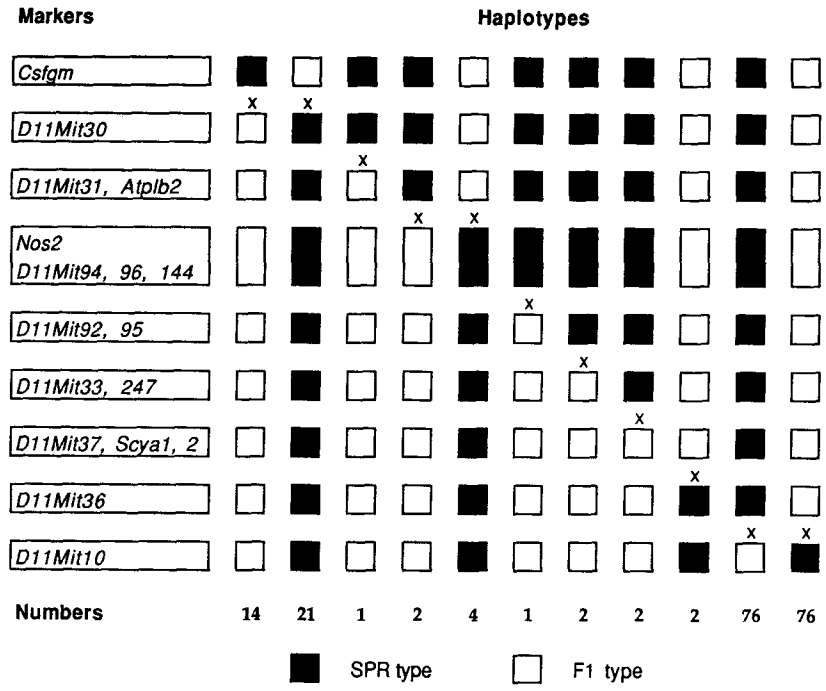

Fig. 3. Distribution of haplotypes in 201 samples of the backcross LS [(SPR × C57Bl/6)F_1 × SPR] which are recombinants between the markers *Csfgm* and *D11Mit 10*. Recombination events are shown as x.

distant by about 2 cM. These data now make it feasible to undertake a positional cloning of *Om* in YACs. The results also show that the locus *Om* has continued to segregate in the BC individuals as a single locus on Chr 11, tightly linked to *Scya1*, suggestive that no other locus is involved. However, it is known that the severity of the DDK syndrome depends on the non-DDK strain used, indicating that there may be several alleles for *Om* or that the genetic background may exert an influence. A physical map of region between *Scya2* and *D11Mit36* is in progress, from which we hope to isolate transcribed sequences. This positional cloning of *Om* should make it possible to define the molecular mechanisms involved in the lethality of DDK × non-DDK embryos. Furthermore, there exists a significant synteny homology between mouse Chr 11 and human Chr 17 in this region (Loosie et al. 1994), suggestive that there maybe a locus *Om* present in humans.

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