potentially explain the discrepancy observed. One obvious difference is the fact that different *Brassica* species and different *S*-alleles were studied. It is, therefore, difficult to make any definitive statements or draw any real comparisons at present, except to say that these interactions appear to be in roughly the same range as some animal receptor–ligand binding affinities.

SCR/SP11 also interacts with SLG

Both labs tested the ability of SCR/SP11 to interact with the other stigmatic component, SLG, as the role of this component in the SI response is rather unclear. Both papers present data that, although not definitive, clearly indicate that SCR/SP11 also interacts with SLG, but not as strongly as to SRK [8,9]. Because both labs, using different approaches, came to the same conclusion, this seems a probable scenario. Furthermore, Takayama's data [9], using cross-linked SP11 to immunoprecipitate SLG, suggest a very close association between SRK and SLG. They propose a model whereby SRK and SLG form a high-affinity receptor complex that interacts with SCR/SP11. The fact that SLG and SCR/SP11 interact is not altogether surprising, because the 'ectodomain' of SRK shares significant homology with SLG; indeed, this was how SRK was identified. However, the biological significance of the interaction between SLG and SCR/SP11 is unclear, especially as SLG is not necessary for the SI response, at least in certain haplotypes [14].

SCR/SP11 can stimulate SRK autophosphorylation

Finally, it has been demonstrated [9] that SP11 can induce autophosphorylation of SRK in an *S*-allele-specific manner. Although it was demonstrated previously that pollen coat proteins can elicit this response [7], this is the first time that it has been established that the pollen *S* receptor itself (and alone) can stimulate phosphorylation of this stigmatic receptor kinase. This is, therefore, an important observation, because it provides insight into the nature of the interaction between SP11 and SRK. It is assumed that the interaction triggers a signalling cascade as a consequence of the *Brassica* SI response.

Conclusion

The demonstration that SCR and SRK interact not only provides a major breakthrough in our understanding of the SI response, but also in our knowledge about receptor–ligand interactions in plant cells. This lays the foundation for a more detailed understanding of receptor–ligand interactions in general. It also will allow a detailed analysis of the signal transduction cascade assumed to be triggered by the SRK–SCR interaction.

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References

1 Lawrence, M.J. (2000) Population genetics of the homomorphic self-incompatibility polymorphisms in flowering plants. *Ann. Bot.* 85(A), 221–226
2 Wheeler, M.J. *et al.* (2001) The molecular and genetic basis of pollen–pistil interactions. *New Phytol.* 151, 565–584
3 Stein, J.C. *et al.* (1991) Molecular cloning of a putative receptor protein-kinase gene encoded at the self-incompatibility locus of *Brassica oleracea*. *Proc. Natl. Acad. Sci. U. S. A.* 88, 8816–8820
4 Takasaki, T. *et al.* (1999) The *S* receptor kinase determines self-incompatibility in *Brassica* stigmas. *Nature* 403, 913–916
5 Schopfer, C.R. *et al.* (1999) The male determinant of self-incompatibility in *Brassica*. *Science* 286, 1697–1700
6 Shiba, H. *et al.* (2001) A pollen coat protein, SP11/SCR, determines the pollen S-specificity in the self-incompatibility of *Brassica* species. *Plant Physiol.* 125, 2095–2103
7 Cabrillac, D. *et al.* (2001) The *S*-locus receptor kinase is inhibited by thioredoxins and activated by pollen coat proteins. *Nature* 410, 220–223
8 Kachroo, A. *et al.* (2001) Allele-specific receptor ligand interactions in *Brassica* self-incompatibility. *Science* 293, 1824–1826
9 Takayama S. *et al.* (2001) Direct ligand–receptor complex interaction controls *Brassica* self-incompatibility. *Nature* 413, 534–538
10 Trontochaud, A.E. *et al.* (2000) CLAVATA3, a multimeric ligand for the CLAVATA1 receptor kinase. *Science* 289, 613–617
11 Gomez-Gomez, L., *et al.* (2001) Both the extracellular leucine-rich repeat domain and the kinase activity of FLS2 are required for flagellin binding and signalling in *Arabidopsis*. *Plant Cell* 13, 1155–1163
12 Torii, U. and Clark, S.E. (2000) Receptor-like kinases in plant development. *Adv. Bot. Res.* 32, 225–267
13 Wang, Z-Y. *et al.* (2001) BRI1 is a critical component of a plasma-membrane receptor for plant steroids. *Nature* 410, 380–383
14 Suzuki, G. *et al.* (2000) Characterization of *Brassica* haplotypes lacking *S*-glycoprotein. *FEBS Lett.* 482, 102–108
15 Franklin-Tong, V.E. and Franklin, C.H. (2000) Self-incompatibility in *Brassica*: the elusive pollen *S* gene is identified. *Plant Cell* 12, 305–308

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QTL for timing: a natural diversity of clock genes

Neeraj Salathia, Kieron Edwards and Andrew J. Millar

Conventional, forward genetics has identified several molecular components of circadian clocks. Many additional loci and genetic interactions have recently been implicated in rhythmic control by a major effort in mapping quantitative trait loci (QTL) in the mouse. Reconciling the QTL with previous results both from QTL and mutagenesis will be a challenge for rhythm researchers.

Most eukaryotes and some prokaryotes have evolved biological clocks that regulate behaviour and physiology rhythmically, in a daily sequence that can anticipate the environmental cycle. The clock is termed 'circadian', meaning 'about daily', because it does not keep exactly 24-hour days. In Nature, daily light and temperature cycles reset the clock and synchronize it with the 24-hour rotation of the Earth. Exactly when the daily round starts, relative to dawn ('phase angle'), and which rhythms occur in what sequence, are probably affected by the array of selective pressures in particular habitats.

Many induced mutations have now been studied to bring some understanding of 'clock genes' (genes that are required to construct the circadian clock) in the five

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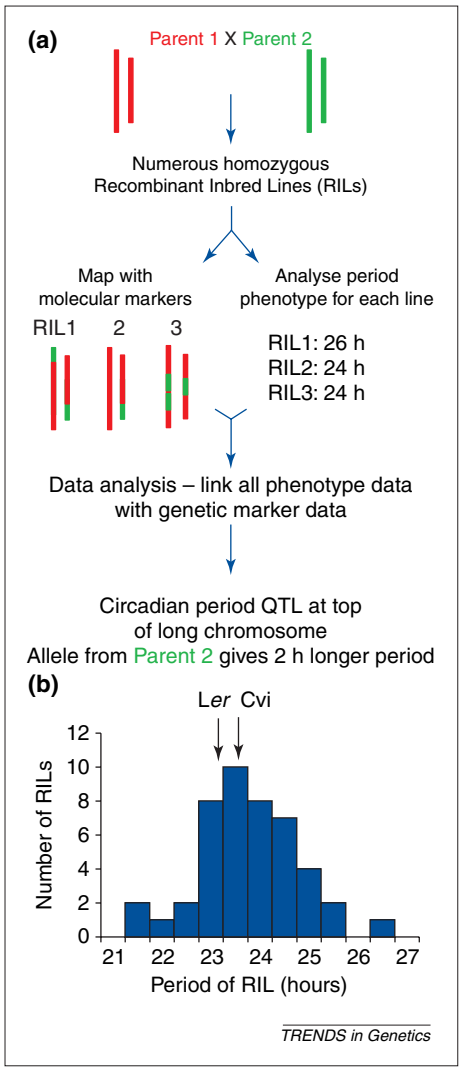


Fig. 1. Quantitative trait loci (QTL) analysis. (a) A simplified flow chart explaining QTL analysis. Parent strains of a hypothetical species with two chromosomes are crossed and a population of inbred lines is produced. The parents need not differ greatly in phenotype [see (b)]. An example of three such recombinant inbred lines is given in the diagram above (RIL1, RIL2, RIL3). Each of these inbred lines is mapped with a set of genetic markers that are polymorphic between the parental strains. Subsequently they are assayed for the phenotype of interest; for example, circadian period (see Box 1 for definition). Phenotype values and genotype data from all the RILs are compiled for analysis in QTL mapping software (often with additional map information). (b) Transgressive segregation of a phenotype compared with parental phenotypes. The period of rhythmic leaf movements of a panel of *Arabidopsis* RILs was assayed in constant light [2]. The parent lines (Cvi and L*er*) are very similar (arrows), suggesting that they each contain balanced, long- and short-period alleles, which recombine in the RILs to give periods over a 5-hour range. No distinct phenotypic classes can be distinguished in the frequency distribution of the progeny, instead the distribution is continuous indicating that the circadian period is controlled polygenically.

most advanced model systems (cyanobacteria, *Neurospora crassa*, *Arabidopsis thaliana*, *Drosophila melanogaster* and mouse). In practice, screens for rhythm mutants are laborious, and only a limited number of mutations can be induced in a single genome, which limits the number of genes that can be identified. Strains, ecotypes and sub-species accumulate many more mutations (so-called natural allelic variation). The variation is so rich that several allelic variants might affect any biological process of interest. This complicates genetic analysis but can also reveal gene interactions that might not be apparent in conventional mutagenesis. Quantitative trait locus (QTL) mapping is used to dissect such polygenic effects. Controversy remains over the relative merits of QTL approaches as opposed to mutagenesis, but a recent analysis of QTL that affect the mouse circadian clock [1] illustrates their potential benefits and limitations.

QTL analysis relies on the basic concept of genetic mapping: the more closely a genetic marker is linked to a clock gene on the chromosome, the more often the marker will segregate with the phenotypic effect of that clock gene. If the alleles of the marker from the two parental strains are distinguishable (e.g. by single nucleotide polymorphism or microsatellite), the marker can be tested to map the clock gene in a population of progeny derived from a cross between strains (Fig. 1). The numerous recombinant progeny that are required can be generated by several strategies. Shimomura *et al.* [1] used the F2 generation from an interstrain cross, which is easy to generate but only lives as long as the individual F2 mice. Recombinant lines that are homozygous at essentially all loci (such as 'recombinant inbred lines'; RILs [2]) are laborious to construct; for example, by 20 generations of full-sibling mating in mice or by eight generations of single-seed descent in *Arabidopsis thaliana*. However, the population is essentially immortal, because it is true-breeding. Each individual within a line has the same genotype, so replication can increase the statistical power of phenotypic tests compared with an F2, where the genotype of each individual is different. Several panels of recombinant inbred (RI) mice are available from The Jackson Laboratory, for example (http://www.informatics.jax.org/).

With recombinant lines in hand, the three prerequisites for a QTL analysis are:
(1) Molecular mapping markers to cover the genome (usually 10–15 cM apart).
(2) The genotype at each marker locus in all the recombinant progeny.
(3) A quantitative measure of the phenotype(s) of each recombinant individual or line.

Several software packages are available to compare the phenotype of each line with its genotype, to identify the chromosomal regions that consistently affect the phenotype (http://www.public.iastate.edu/~mmalek/Qtllinks.html). The differences in the underlying statistical methods can be significant but obscure to the non-specialist, not least because they are the subject of active research. Not all of the programs are user-friendly, but interfaces are periodically revised, so this situation should improve.

Once identified, such chromosomal regions are 'provisional QTL': they should contain a gene or genes of interest, but they are identified by statistical associations. Usually, no recombinant line carries only a single QTL, rather their phenotypes involve interactions among several genes. Variation owing to environmental effects can also be significant, because the effect of each QTL is often relatively small. Understanding QTL phenotypes is therefore more complex than the simple comparison of an induced mutant to its wild-type parent.

A large, recent study from the Takahashi group [1] identified 14 QTL involved in circadian timing, in a panel of 196 F2 mice derived from a cross between the most commonly used lab strains C57BL/6 and BALB/c. Successful QTL analysis does not require dramatic phenotypic differences between the parents (Fig. 1b), indeed these parental strains differ in circadian period by only 0.6 hours, although in phase angle they differ by more than 3 hours (see Box 1 for definition of circadian terms). They have the major advantage that many polymorphic markers for genetic mapping have been described. The rhythmic, running-wheel activity of each mouse was measured during a week of entrainment in 12 hour light–dark cycles followed by three weeks in constant darkness. The large panel of lines screened and the long assay period allowed for the accurate measurement of five different aspects of circadian timing (Box 1). Genetic maps of each individual were established using simple sequence length polymorphism (SSLP) markers. Standard software and

additional methods developed by the group were used to identify five provisional QTL for period (2 QTL), phase angle (2 QTL) and amplitude (1 QTL). One period QTL on chromosome 4 was linked to a QTL identified previously in a cross of C57BL/6 to another strain, DBA/2 [3].

Further QTL were identified through their effects as interacting pairs: these cases include epistatic interactions, in which the QTL effect of one locus is only detected in the presence of a particular allele at another locus. Four of the five 'single-locus' QTL were also involved in such interactions, in addition to nine loci that had little effect alone (and might therefore be difficult to find by mutagenesis). The pairs of interacting loci affected the circadian period (1 pair), phase angle (3 pairs), amplitude (1 pair), total activity level (1 pair) and dissociation of rhythmic activity from one episode per day into several (1 pair). The QTL explained a substantial proportion of the variance: 37% of the variance in phase angle was accounted for by the QTL and their interactions, for example.

Previous studies have used both F2 and RI mice to identify circadian clock QTL. RI panels derived from BALB/c × C57BL/6 and C57BL/6 × DBA/2 crosses were tested for circadian period in constant darkness, identifying over 18 putative QTL (e.g. Refs [3,4]). The number of lines tested was much smaller than the F2 population of Shimomura *et al.* and the range of periods in both panels of recombinant progeny was also small; reliable data (for both map and phenotype) should nonetheless enable QTL to be identified from only small differences in phenotype. Some QTL were common to both panels, however other unique loci showed the value of studying more than one panel. Other RI panels or F2 crosses have revealed QTL for further aspects of circadian timing, such as the phase angle [5] and rhythmic amplitude [6]. Comparisons among these studies can be difficult, owing to the variety of statistical methods, potential environmental influences and the inability to replicate F2 populations exactly for independent testing.

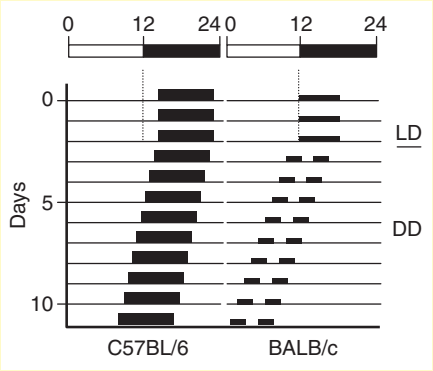
To move beyond such problems, the construction of congenic lines is now essential. Congenic lines differ from a standard, inbred parent only at the single locus responsible for the differential phenotypic effect on the trait, initially indicated by a provisional QTL. Phenotypic tests on congenic lines firstly confirm the location and effect of the provisional QTL as in Ref. [7] and secondly allow the identification of the underlying genes, by fine-mapping or candidate gene approaches. The availability of genomic DNA sequence from several mouse strains in the near future will greatly facilitate such efforts in mice. Similar methods in *Arabidopsis*, where the strains are known as near isogenic lines (NILs), have confirmed circadian clock QTL in this species [2] and underpinned efforts to clone QTL that affect seed dormancy, photoperiodic timing [8] and photoreceptors [9].

A key question remains as to whether QTL analysis and mutagenesis will ultimately identify the same genes. Nine mouse genes have been implicated in circadian timing by molecular genetics and/or mutagenesis. The Takahashi group mapped the last five of these [1], but only one QTL (for dissociation) was closely linked to a candidate gene identified by mutation, casein kinase 1ε. One view holds that 'central' genes are too important to brook allelic variation and so variants would be eliminated by selection, so they cannot be identified as QTL. However, 'central' genes controlling the fly clock [10], and photoperception [9] and flowering time in plants [8,11] clearly do vary. Comparing past results from mutagenesis and QTL analysis reveals their practical constraints. Past QTL analysis could only have identified genes that happened to vary between parental strains that were selected in part for convenience of genetic mapping. In the mouse F2 panel, the variation in phase angles was much greater than the range of periods [1], suggesting that this study might be more informative about phase angle. Previous 'clock' screens after mutagenesis have concentrated on period mutants, however, so there is less known about the genetic control of phase angle. Parental strains will increasingly be selected for their differing phenotypes, focusing research effort where natural variation is most likely to be identified [9].

There is much scope for using QTL to understand the complexity of the clock, some of which is likely to evolve in response to selective pressures. *FLOWERING LOCUS C* of *Arabidopsis,* for example, is an important determinant of flowering time [11] but had no known clock function until it was identified as a period QTL [2]. Ambient light and temperature vary among habitats and have major effects on circadian rhythms,

Box 1. The rhythmic characteristics used for QTL analysis

The characteristics used for QTL analysis by Shimomura *et al.* [a] can be illustrated on an actogram, in which each day's running wheel activity (solid bar) is plotted beneath that of the preceding day. In practice, a variety of software packages were used for data analysis, in addition to visual inspection of the data. The phase angle is the time between the actual onset of darkness (vertical line) in a light–dark cycle (LD) and the activity onset, which is predicted from the rhythm in constant darkness (DD) to avoid direct effects of light. All other measurements are taken in constant darkness. The circadian period in mice is the mean time between activity onsets. The amplitude of the rhythm is the difference between the peak and the trough rate of locomotor activity. The activity level is the mean number of running-wheel rotations per day. Dissociation measures the occurrence of multiple, discrete bouts of activity per cycle as opposed to a single activity episode per cycle. The right-hand panel in the cartoon depicts the shorter period, earlier phase angle, lower activity level and dissociation that are typical of the BALB/c strain, compared with C57BL/6 in the left-hand panel.



Reference

a Shimomura, K. *et al.* (2001) Genome-wide epistatic interaction analysis reveals complex genetic determinants of circadian behavior in mice. *Genome Res.* 11, 959–980

so they are likely to have driven adaptive change. We have recently located new circadian QTL in *Arabidopsis*, several which are specific for particular temperature regimes (K. Edwards and A.J. Millar, unpublished). QTL analysis in mice might thus reveal pleiotropic genes that are involved in the mouse clock. These might well include 'central' clock genes that are otherwise difficult to identify. Phenotypic testing under various environmental conditions can increase the number of genes recovered. The method can be efficient: testing 196 F2 individuals [1] is not such a large number, compared to a conventional mouse screen after mutagenesis. Strain collections with detailed behavioural and habitat data are still required to complete the causal chain from temporal ecology to the molecular mechanism of the clock, but the supporting tools are being moved into place [12].

References

1 Shimomura, K. *et al.* (2001) Genome-wide epistatic interaction analysis reveals complex genetic determinants of circadian behavior in mice. *Genome Res.* 11, 959–980
2 Swarup, K. *et al.* (1999) Natural allelic variation identifies new genes in the *Arabidopsis* circadian system. *Plant J.* 20, 67–77
3 Hofstetter, J.R. *et al.* (1999) Provisional QTL for circadian period of wheel running in laboratory mice: quantitative genetics of period in RI mice. *Chronobiol. Int.* 16, 269–279
4 Hofstetter, J.R. *et al.* (1995) Quantitative trait loci (QTL) for circadian rhythms of locomotor activity in mice. *Behav. Genet.* 25, 545–556
5 Suzuki, T. *et al.* (2000) Mapping quantitative trait loci for circadian behavioral rhythms in SMXA recombinant inbred strains. *Behav. Genet.* 30, 447–453
6 Toth, L.A. and Williams, R.W. (1999) A quantitative genetic analysis of locomotor activity in CXB recombinant inbred mice. *Behav. Genet.* 29, 319–328
7 Mayeda, A.R. and Hofstetter, J.R. (1999) A QTL for the genetic variance in free-running period and level of locomotor activity between inbred strains of mice. *Behav. Genet.* 29, 171–176
8 El-Assal, S.E. *et al.* (2001) A QTL for flowering time in *Arabidopsis* reveals a novel allele of CRY2. *Nat. Genet.* 29, 435–440
9 Maloof, J.N. *et al.* (2001) Natural variation of light sensitivity in *Arabidopsis*. *Nat. Genet.* 29, 441–446
10 Sawyer, L.A. *et al.* (1997) Natural variation in a *Drosophila* clock gene and temperature compensation. *Science* 278, 2117–2120
11 Michaels, S.D. and Amasino, R.M. (2001) Loss of FLOWERING LOCUS C activity eliminates the late-flowering phenotype of FRIGIDA and autonomous pathway mutations but not responsiveness to vernalization. *Plant Cell* 13, 935–941
12 Mauricio, R. (2001) Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology. *Nat. Rev. Genet.* 2, 370–381

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Meeting Report

Five years of vector service for gene therapy

Mauro Mezzina and Olivier Danos

The Gene Vector Production Network Conference (http://www.genethon.fr/gvpnconf.html) was held at Genocentre, Evry, France, from 4 to 5 October 2001.

The Gene Vector Production Network (GVPN; http://www.genethon.fr/gvpn) is a coordinated service for the production of reagents for gene transfer experiments (viral vectors, plasmids and cell lines) for the scientific community. It started in 1997, following an initiative of the Association Française contre les Myopathies (AFM) to encourage the development of gene therapy for genetic diseases. The objectives of the meeting were to assess progress, and to allow GVPN users to meet, develop collaborations and plan future strategies. Abstracts are available on the conference website.

GVPN material has been used in a wide range of projects aimed at the treatment of genetic, degenerative, auto-immune and virally induced diseases, and cancer. In addition, there has been further development of gene transfer technology; scaling up vector production, purification and quality control, routes of administration, transgene expression and immune response.

The first gene therapy successes

The first success was achieved at the Necker Hospital in Paris, with treatment of four children suffering from X-linked Severe Combined Immune Deficiency (SCID-X). Patients were auto-grafted with hematopoietic stem cells (HSC) transduced with a retrovirus carrying the wild-type γC gene. This restored normal immune function, all clinical symptoms disappeared, and the children are healthy to date [1]. Marina Cavazzana-Calvo (Necker Hospital, Paris, France) envisages performing additional trials using a similar protocol with other SCID children presenting alterations in the *RAG-1* and *RAG-2* genes, which encode two other factors involved in the immune disorder.

Naomi Taylor (IGM-CNRS, Montpellier, France) is developing a similar protocol for SCID patients with altered *ZAP-70*, another factor involved in the disease. Re-population with $CD4^+$ and $CD8^+$ differentiated cells occurred normally in *zap-70*$^{-/-}$ mice grafted with HSC transduced with the *zap-70* gene, although the characterization of the resulting phenotype is not yet complete. Thus it seems that retroviral vectors are efficient vehicles for the transfer of therapeutic factors into hematopoietic cells and could be used to treat other immune and hematological disorders.

Other approaches to treatment of genetic diseases

Guerrino Meneguzzi (INSERM-CHU, Nice, France) was able to produce laminin-β3 (LB3) in LB3-deficient keratinocytes from junctional epidermolysis bullosa (JEB) patients [2]. The keratinocytes were transduced with the wild-type *LB3* gene using retroviral vectors, which transduced almost 100% of cells, confirming the efficiency of this vector system for epidermal stem cells. The feasibility of treating genetic skin diseases by grafting engineered epidermal stem cells on to human patients is currently under investigation in dog models of JEB.

Marc Peschanski (INSERM, Creteil, France) reported on a Phase I–II trial in which he implanted

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