36 Buizza, A. *et al.* (1980) *Exp. Brain Res.* 39, 165–176
37 Nakayama, K. (1981) *Vision Res.* 21, 1475–1482
38 Dijkstra, T.M.H. *et al.* (1995) *Vision Res.* 35, 453–462
39 Van den Berg, A.V. and Collewijn, H. (1986) *Vision Res.* 26, 1209–1222
40 Ungerleider, L.G. and Mishkin, M. (1982) in *Analysis of Visual Behavior* (Ingle, D.J., Goodale, M.A. and Mansfield, R.J.W., eds), pp. 549–586, MIT Press
41 Maunsell, J.H.R. and Van Essen, D.C. (1983) *J. Neurosci.* 3, 2563–2586
42 Koenderink, J.J. and Van Doorn, A.J. (1975) *Optica Acta* 22, 773–791
43 Saito, H. *et al.* (1986) *J. Neurosci.* 6, 145–157
44 Lagae, L. *et al.* (1994) *J. Neurophysiol.* 71, 1597–1626
45 Duffy, C.J. and Wurtz, R.H. (1991) *J. Neurophysiol.* 65, 1329–1345
46 Motter, B.C. and Mountcastle, V.B. (1981) *J. Neurosci.* 1, 3–26
47 Orban, G.A. *et al.* (1992) *Proc. Natl Acad. Sci. USA* 89, 2595–2599
48 Allman, J., Miezin, F. and McGuinness, E. (1985) *Perception* 14, 105–126
49 Maunsell, J.H.R. and Van Essen, D.C. (1983) *J. Neurophysiol.* 49, 1148–1167
50 Roy, J.P. and Wurtz, R.H. (1990) *Nature* 348, 160–162
51 Bradley, D.C., Qian, N. and Andersen, R.A. (1995) *Nature* 373, 609–611
52 Andersen, R.A. (1987) in *Handbook of Physiology* (Section 1, Vol. 5) (Brookhart, J.M. and Mountcastle, V.B., eds), pp. 483–518, American Physiological Society
53 Komatsu, K. and Wurtz, R.H. (1988) *J. Neurophysiol.* 60, 580–603
54 Sakata, H., Shibutani, H. and Kawano, K. (1980) *J. Neurophysiol.* 43, 1654–1672
55 Kawano, K., Sasaki, M. and Yamashita, M. (1984) *J. Neurophysiol.* 51, 340–351
56 Thier, P. and Erickson, R.G. (1992) *Eur. J. Neurosci.* 4, 539–553
57 Berman, N., Blakemore, C. and Cynader, M. (1975) *J. Physiol.* 246, 595–615
58 Hoffmann, K.P. (1982) in *Functional Basis of Ocular Motility Disorder* (Lennerstrand, G., Zee, D.S. and Keller, E.L., eds), pp. 303–311, Pergamon
59 Grasse, K.L. (1994) *Vision Res.* 34, 1673–1689

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Neuronal networks for induced '40 Hz' rhythms

John G.R. Jefferys, Roger D. Traub and Miles A. Whittington

A fast, coherent EEG rhythm, called a gamma or a '40 Hz' rhythm, has been implicated both in higher brain functions, such as the 'binding' of features that are detected by sensory cortices into perceived objects, and in lower level processes, such as the phase coding of neuronal activity. Computer simulations of several parts of the brain suggest that gamma rhythms can be generated by pools of excitatory neurones, networks of inhibitory neurones, or networks of both excitatory and inhibitory neurones. The strongest experimental evidence for rhythm generators has been shown for: (1) neocortical and thalamic neurones that are intrinsic '40 Hz' oscillators, although synchrony still requires network mechanisms; and (2) hippocampal and neocortical networks of mutually inhibitory interneurones that generate collective 40 Hz rhythms when excited tonically.

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J.G.R. Jefferys is at the Dept of Physiology, The Medical School, University of Birmingham, Birmingham, UK B15 2TT. Miles A. Whittington is at the Neuronal Networks Group, Dept of Physiology and Biophysics, St Mary's Hospital Medical School, Imperial College, London, UK W2 1PG. Roger D. Traub is at the IBM Research Division, T.J. Watson Research Center, Yorktown Heights, NY 10598, USA, and the Dept of Neurology, Columbia University, New York, NY 10032, USA.

FAST, GAMMA RHYTHMS have been implicated in higher cognitive function. They are also known as '40 Hz' rhythms, but actually range from 30 to 100 Hz and might vary in frequency during a response. The 20–100 Hz range we consider here overlaps with the beta band (15–30 Hz) of the EEG, but we will ignore the finer points of EEG classification. The natural history and functional roles of synchronous gamma oscillations have been reviewed recently[1–3], and so will be considered only briefly.

Gamma rhythms occur in humans and other mammals following sensory stimuli, often in brief runs. 'Induced rhythms' at 50–60 Hz were first described in the olfactory bulb by Adrian[4], and have since been identified in the olfactory cortex[5], visual cortex[3,6–9], auditory cortex[10,11], somatosensory cortex[12] and motor cortex[13–15]. Gamma oscillations also occur in the hippocampus[16,17], where the link with external sensory stimuli is less direct, but might still exist in the form of multimodal inputs received from higher-order sensory cortices. Hippocampal gamma rhythms tend to occur during the theta band (4–12 Hz) of the EEG, which is a prominent feature of the hippocampus *in vivo*[16,18], especially during exploration.

In humans the auditory response includes brief '40 Hz transient responses'[19,20], which increase when the subject pays attention, and which disappear with loss of consciousness during anaesthesia[21]. Repetitive auditory stimulation at ~40 Hz generates a large '40 Hz steady-state response'[22]. Recordings of brain magnetic activity (magnetoencephalograms or MEGs) in humans suggest that gamma rhythms can be very widespread[23], during both waking and dream states. Other MEG measurements in humans suggest that gamma rhythms might be organized to sweep across the whole brain, perhaps providing 'temporal binding...into a single cognitive experience'[24].

Neuronal firing

Single-unit recordings *in vivo* have revealed much about the events or features to which neurones respond. Individual neurones do not detect their preferred sensory features in isolation, but form part of neuronal networks whose emergent properties define the feature-detection properties of the cortical column. In the visual system, it used to be thought that successive hierarchies of neurones encoded progressively more-complex features of

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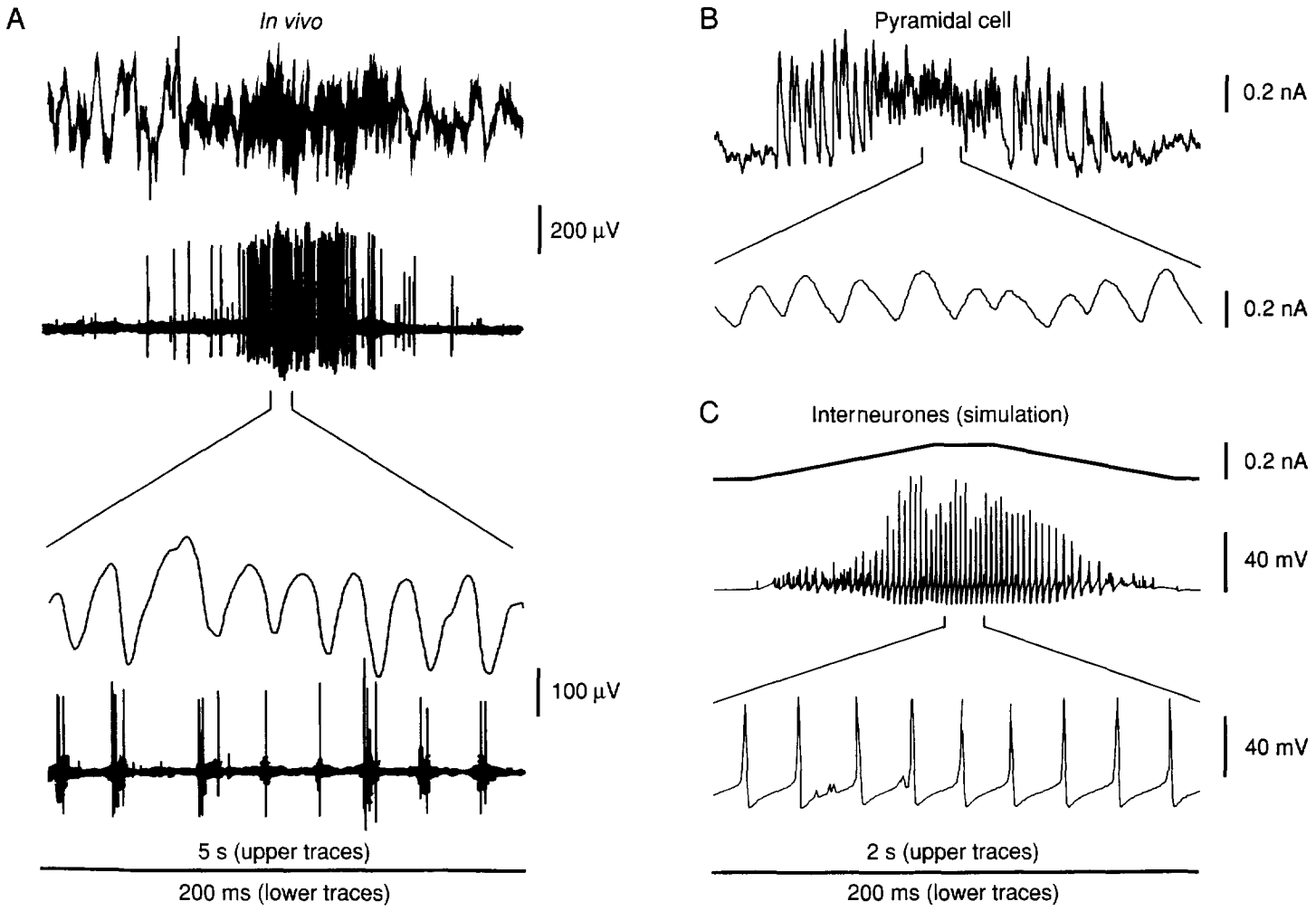

Fig. 1. *40 Hz oscillations.* **(A)** *Recordings from the cat visual cortex show synchronous oscillations in the local-field potential (top trace) and multi-unit recordings (second trace) in response to a moving-bar visual stimulus. These have been expanded below to reveal an oscillation in the local-field potential of 40 Hz and the phase-locked discharge of local neurones.* Adapted, with permission, from Ref. 3. **(B)** *A recording from a CA1 pyramidal cell in a rat hippocampal slice following a pulse of glutamate shows that an oscillation of ~40 Hz can be induced* in vitro. *The expanded portion of the trace represents the same timescale as in* A *(timescales are the same for* B *and* C*).* **(C)** *The hippocampal ~40 Hz oscillation is driven by the network of inhibitory neurones. Illustrated is a simulation of the application of an inward ('glutamate') current (top trace) to a network of 16 inhibitory neurones which induced a ~40 Hz discharge evident in the mean membrane potential of these neurones.*

objects. This scheme, however, is inflexible and inefficient: conjunctions of more and more combinations of 'low-level' features are needed to define progressively 'higher-level' features. It is difficult to imagine how such a scheme copes with the vast range of objects we are able to recognize (the 'combinatorial problem'[3]), and with the many entirely unprecedented objects found in the modern world. For example, frisbees have no obvious precedent in the world in which our ancestors evolved. (Although, a recent visit to the Pitt Rivers Museum in Oxford revealed that circular quoits do have a long history on the Indian subcontinent as a kind of throwing knife, but these were made of metal, not brightly coloured plastic!) Yet we are able effortlessly to distinguish a flying frisbee against a complex background, even though the different, widely separated, cortical areas that detect the frisbee's shape, colour or movement also detect the features of the background. All this information can then be linked efficiently to the motor system to allow us to catch the frisbee. A plausible idea for how we accomplish such tasks is that transient 'binding' functionally links the often discontinuous cortical networks needed to analyse the many features that make up real objects[3], and conversely, 'scene segmentation' functionally separates the networks encoding different objects from one another and from the background.

The problems of binding, segmentation and the role of synchronous oscillations were clearly identified by von der Malsburg[25] when considering the 'cocktail-party effect', where we are able to listen to one speaker despite the background babble. Much of the recent interest in gamma rhythms centres on the visual system[3,6–9]. Single- and multi-unit discharges and local-field potentials (Fig. 1) can be synchronized at frequencies in the gamma band between visual cortical areas separated by long distances (as long as those areas are stimulated by, and selective for, the same object). The repeated, synchronized firing of neurones in co-stimulated areas provides, in principle, a solution to the binding problem. This concept might generalize beyond the specific sensory cortices to association areas and the hippocampus, where information from different sensory modalities converge and might be bound into multimodal entities or perceptions. The key point is that the gamma oscillation is not proposed to represent information itself, but rather to provide a temporal structure for correlations in the neurones that do encode specific information[3,26,27].

The general significance of this mechanism is controversial. Not everyone finds unit correlations in the gamma band, for example, in some of the experiments performed on the visual cortex of the monkey[28,29]. This could be related to choices of stimuli and recording

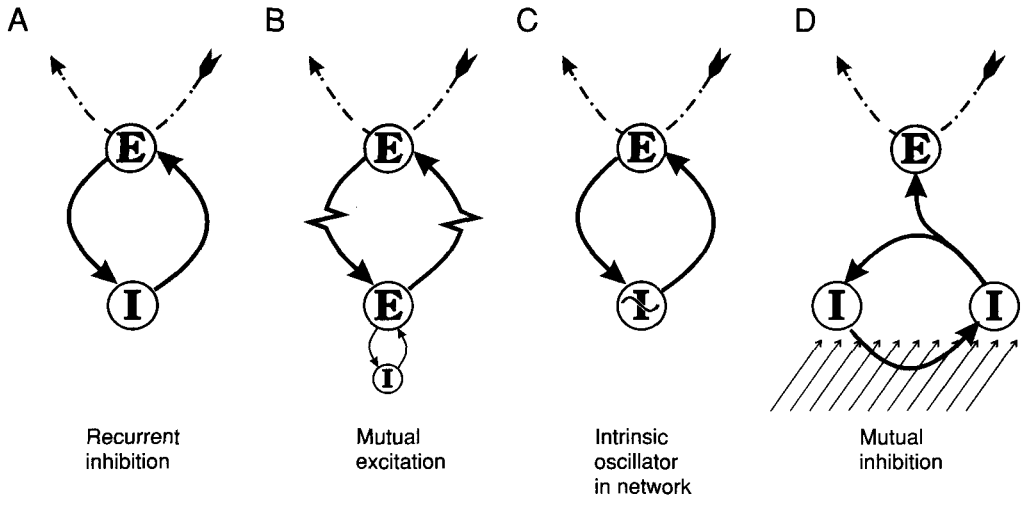

Fig. 2. ***Simplified representations to illustrate the essential features of several mechanisms proposed to be involved in the generation of gamma oscillations.*** *In each case, E (excitatory) and I (inhibitory) represent networks of neurones that are mutually connected, the continuous lines indicate the key connections for their respective mechanisms, and the dot–dash arrow indicates the flow of specific information through the network.* **(A)** *illustrates the recurrent inhibitory loop model proposed by Freeman* et al.[5] *Computer theoretical analysis has subsequently shown that mutual excitation is required, but that mutual inhibition is not.* **(B)** *shows a similar model in which the time delays along the axons coupling groups of excitatory neurones play a key role*[33]*, and which also receives contributions from recurrent inhibition.* **(C)** *proposes that neurones with intrinsic-oscillator properties can impose their own rhythm on the synaptic network in which they are embedded*[34–37]*.* **(D)** *represents our own model of gamma oscillations in the hippocampus, in which interneurones are tonically excited (thin arrows) so that they will fire at a rate >40 Hz. The divergent inhibitory connections between these neurones result in synchronized inhibition across the population. When this decays, the neurones will discharge due to the tonic excitation that drives the rhythm*[38] *imposing a rhythm of about 40 Hz. Notice that in its simplest form* (D) *separates the role of the oscillator (or clock) and the processor.*

sites, or to technical differences (gamma rhythms can occur in brief bursts with a considerable jitter in the frequency[27], so any correlation could conceivably be smudged out when measurements are averaged during 0.5 s runs of an EEG, or 20 cycles at 40 Hz; Ref. 28). However, the reasons for these discrepancies remain unresolved.

Coherent rhythms might have other functions. One idea is that they provide a timing reference for a neural code that depends on the phase relationship of individual neurones with the reference oscillation. The stronger the excitation to an individual neurone, the earlier in the cycle it will fire. Thus neurones that fire at similar phases in the rhythm will have received similar intensities of input which might be used, for example, to lock their outputs together for a more effective summation. This hypothesis was proposed for theta rhythms in the hippocampus[30], and also, more recently, for gamma rhythms[31].

The role of gamma rhythms is unknown. They might be central to our cognitive function, be fundamental to the neural code, have some entirely different role, or simply be an epiphenomenon[32] with no deep meaning. We believe that one key step to resolving these issues is to understand the cellular and network mechanisms that generate gamma rhythms, and to develop pharmacological tools that will allow us to probe their roles *in vivo*.

What drives the gamma rhythm?

The original models for binding and segmentation introduced the idea that neurones that oscillated together also worked together[25], reflecting that synchronization is a more important factor than a narrow bandwidth[3,27]. Although single episodes of neuronal synchronization might occur by chance, repeated synchronization is much less likely to do so. At the cellular level repeated synchronization could promote temporal summation at active synapses.

Several theories exist for the generation of gamma oscillations in various parts of the brain, which all need further experimental testing. It is possible that gamma oscillations arise by different mechanisms in different parts of the brain, and that several mechanisms can combine in individual regions. Figure 2 shows highly simplified representations of some of the components of these different mechanisms, which we will consider below in roughly chronological order.

Feedback loops between excitatory and inhibitory neurones

(The main regions implicated include: the olfactory bulb, the piriform cortex, the entorhinal cortex and the primary visual cortex.) Freeman and colleagues developed a model for induced rhythms in several olfactory structures, which proposed that synchronous oscillation is generated by a feedback loop between excitatory and inhibitory neurones[5]. They proposed that some mutual connectivity was also required within the pools of both excitatory and inhibitory neurones to stabilize the oscillations. Ermentrout[39] has shown that mutual excitation amongst the excitatory neurones is necessary for stable oscillations to be generated by a recurrent inhibitory loop. However, our recent simulations suggest that other conditions might suffice for stable oscillations (R.D. Traub, unpublished observations).

Freeman *et al.*[5] predicted that inhibitory cells should lag behind the excitatory cells by a quarter of a cycle (6.5 ms at 40 Hz). Experimental support came from single-unit and EEG recordings *in vivo* from the olfactory bulb, anterior olfactory nucleus, prepiriform cortex and entorhinal cortex[5]. The signals fell into two groups: one set fired in phase with the gamma EEG, and one either led or lagged the gamma EEG by a quarter of a cycle. Unfortunately these measurements cannot identify the types of neurones in each group. In contrast, hippocampal interneurones recorded during the gamma EEG fire in phase with pyramidal cells[16]. This is predicted by our inhibitory network model (see below), both when isolated from the excitatory network[38], and when connected with pyramidal cells (R.D. Traub *et al.*, unpublished observations). Why the hippocampal and the (superficially similar) olfactory cortical circuitry should differ remains unclear[5].

Wilson and Bower made similar models of the piriform cortex[33] and the primary visual cortex[32]. The geometric structure of these models differed, but the essential idea in both was that the amplitude and the frequency of coherent 30–60 Hz oscillations, elicited by afferent volleys, were determined (or 'tuned') by a fast-feedback inhibitory loop (Fig. 2A). Essentially, if the stimulus is appropriate (not too strong), enough activity in the recurrent excitatory connections between pyramidal cells persists after the recurrent inhibition

wanes in order to re-excite the pyramidal-cell population. In the case of the piriform-cortex model, they showed that the time constant of inhibition 'tuned' the frequency of the gamma rhythm, so that longer time spent open for the chloride channels resulted in slower rhythms (and also a loss of power).

In their model of the primary visual cortex Wilson and Bower[32] note, in passing, that local mutual inhibition between the interneurones 'improved frequency locking and produced auto- and cross-correlations with more pronounced oscillatory characteristics'. This differs from the central role of similar connections in the generation of gamma rhythms in the hippocampus, where they were both necessary and sufficient[38,40].

In the visual-cortex model, horizontal pyramidal cell axons were essential for long-range (>1 mm) cross-correlations[32]. These had zero phase lag as long as the excitatory postsynaptic potentials (EPSPs) that they generated were not too strong. Stronger EPSPs result in phase lags consistent with delays in axonal conduction, while weaker EPSPs were reminiscent of other kinds of loosely coupled oscillators. In both the visual-cortex and the piriform-cortex versions of this model, gamma rhythms arose from interactions between networks of excitatory neurones, could depend on the conduction velocities of intrinsic cortical connections (Fig. 2B), and were tuned by the time constants of excitatory and inhibitory synapses. We are not aware of any attempts to dissect these complex interactions experimentally; in particular, an investigation of the effects of conduction delays on cortical oscillations would be instructive.

Intrinsic oscillations in individual neurones

(The main regions implicated include the thalamus and the neocortex; see Fig. 2C.) Neurones in many parts of the brain have the intrinsic capacity to oscillate at about 40 Hz. Space does not permit an exhaustive review of intrinsic oscillators; here we outline one or two relevant cases. For example, several types of neurones in the thalamocortical system such as the reticular[34] and intralaminar[35] neurones do so. In the neocortex itself, examples of intrinsic cellular oscillators include: sparsely spiny, layer-4 neurones[36], about 20% of long-axon projection neurones in layers 5 and 6 (Ref. 37), and 'chattering cells' (cells that fire brief trains of action potentials at 200 Hz about 40 times a second), which were recently reported *in vivo*[41].

Slice studies revealed that oscillations of 40 Hz in sparsely spiny neurones in the frontal cortex are generated by persistent, voltage-dependent Na^+ currents and delayed voltage-dependent rectifier currents[36]. Other frontal-cortex neurones use fast persistent Na^+ currents, leak and slow non-inactivating K^+ currents to generate oscillations of 4–20 Hz (Ref. 42). Various models suggest that similar mechanisms can generate oscillations of 40 Hz (Ref. 43). At least some cortical neurones with intrinsic oscillator mechanisms project to contralateral areas, and to the thalamus, providing routes for long-range synchronization of these oscillations[37]. The existence of cells with intrinsic oscillations at ~40 Hz does not in itself explain the synchronization of local populations of neurones, but it is likely to pace population rhythms when the neurones are suitably coupled by chemical or electrical synapses or both[44].

Networks of inhibitory neurones

(The main regions implicated include the hippocampus and the parietal neocortex; see Fig. 2D.) We have recently proposed a new model of gamma rhythms

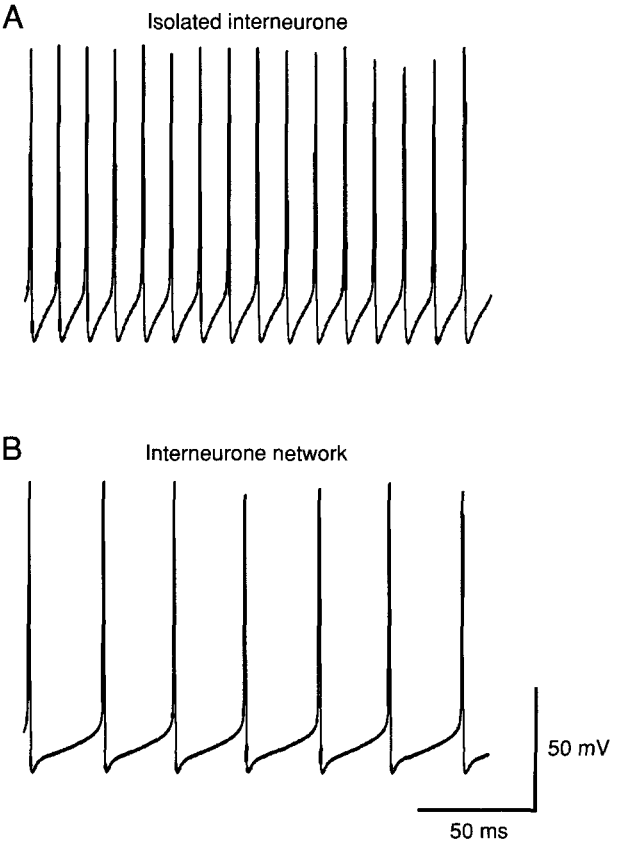

Fig. 3. ***Inhibitory neuronal networks generate gamma oscillations.*** **(A)** *Computer simulation of the brisk excitation of an isolated inhibitory interneurone by an injection of current to mimic the activation of metabotropic glutamate (mGlu) receptors.* **(B)** *The same inhibitory interneurone as part of a network of inhibitory neurones coupled by fast, $GABA_A$-mediated inhibitory postsynaptic potentials (IPSPs). Its response to mGlu receptor activation is now sculpted into an oscillation of 33 Hz by synchronized IPSPs generated by the inhibitory network.*

based on experiments and computer simulations on the hippocampal slice (Fig. 1B,C). Essentially, when networks of inhibitory neurones are tonically excited, they tend to entrain each other into rhythmic firing through their mutual inhibitory connections. Figure 3A shows a computer simulation of the effect of a depolarizing current in an isolated interneurone. The rapid discharge becomes organized into a rhythmic pattern of ~40 Hz when the interneurone is synaptically coupled to a network of similarly activated interneurones (Fig. 3B). In effect the rhythm is sculpted from the tonic discharge by synchronous inhibitory postsynaptic potentials (IPSPs). The experimental evidence for this model is that synchronous IPSPs at frequencies in the gamma band occur in hippocampal and neocortical slices where all ionotropic glutamate-receptor containing synapses are blocked. In these experiments, interneurones are excited by the activation of metabotropic glutamate (mGlu) receptors. The experimental blockade of fast EPSPs excludes models that depend on these (Fig. 2A–C). We are not aware of hippocampal neurones that oscillate preferentially at 40 Hz, and our computer simulations show that such intrinsic oscillators are not necessary for gamma oscillations in a neuronal network. In this model, what is necessary is tonic excitation of the interneurones (for example, from metabotropic or NMDA receptors). Fast EPSPs might be superimposed on the tonic excitation, but they are not required, as shown by the original experiments[38].

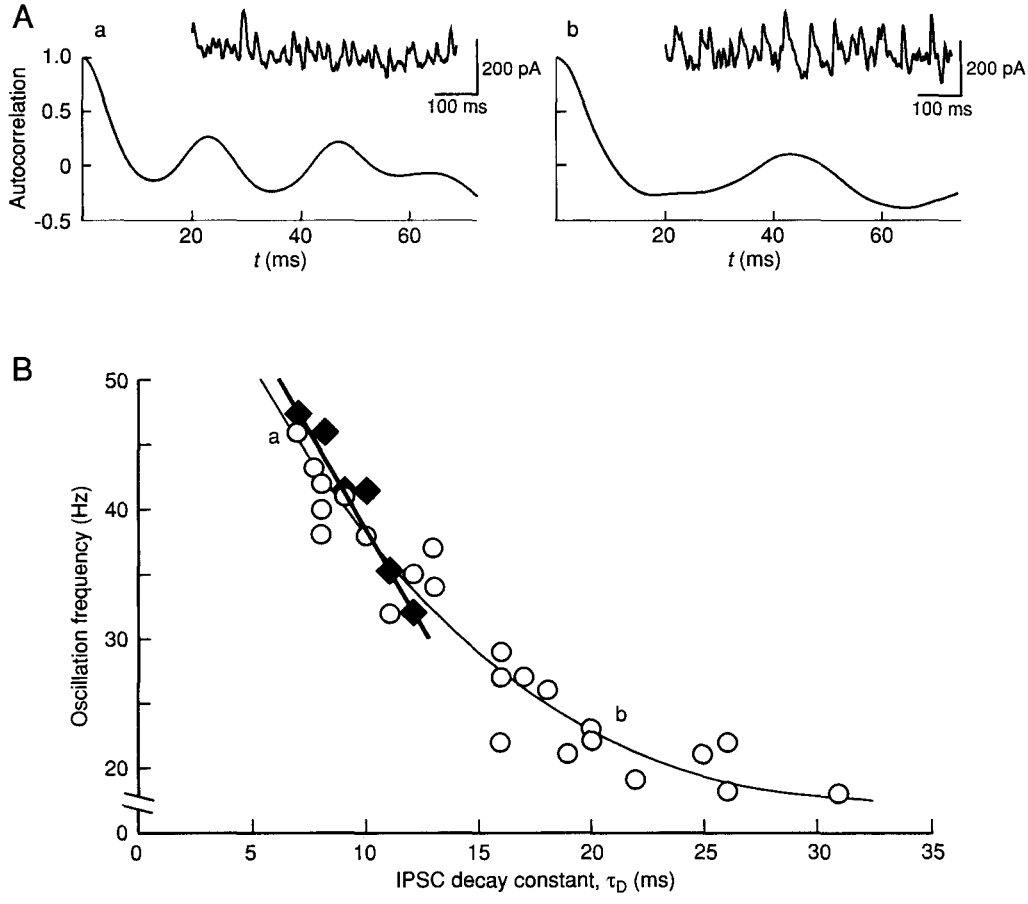

Fig. 4. ***The frequency of oscillation in the inhibitory neuronal network is a function of the decay constant of the inhibitory postsynaptic current (IPSC).*** **(A)** *shows autocorrelations of voltage-clamp recordings from inhibitory interneurones in stratum oriens made during an application of glutamate in the presence of drugs to block ionotropic glutamate receptors.* (a) *Prior to addition of 20 μM pentobarbital, the network oscillated at 22.7 ms [44 Hz; IPSC decay constant (τ_D) was 9.1 ± 0.4 ms], which is faster than pyramidal cells which have a τ_D of 22.4 ± 0.8 ms.* (b) *After equilibration with pentobarbital the period slowed to 44.5 ms (22 Hz; τ_D reached >30 ms).* **(B)** *Measurements made of both network frequency and τ_D (open circles) during the wash-in of 2 μM pentobarbital reveal a close relationship, which matches that predicted by computer simulations (filled diamonds). More recent computer simulations match the non-linearity found at lower frequencies and the upper and lower limits to the synchronous network oscillations, following an increase in the connectivity of the simulated network*[40]. Figure adapted, with permission, from Ref. 38.

The frequency of the oscillation is controlled, in part, by the time constant of the fast $GABA_A$-mediated IPSP. Computer simulations have predicted, and experiments have confirmed, that drugs that slow the decay of the IPSP (for example, barbiturates), also slow the frequency of the oscillation (Fig. 4). (In 1950, Adrian made a similar observation on the olfactory bulb when he noted that frequencies of induced oscillations in the olfactory bulb differed between urethane-induced and barbiturate-induced anaesthesia, at around 50 and 15 Hz respectively[4].) In a more extensive exploration of the control of these synchronous, inhibitory gamma oscillations, we find that they can exist over a range of 20–70 Hz, and that they desynchronize outside of this range[40]. The oscillations speed up, in both experiment and simulation, with an increased excitatory drive, a shortened inhibitory postsynaptic current (IPSC) decay constant (τ_{GABA}), or a decreased IPSC amplitude. The recordings also show that at least two classes of interneurone participate in this activity: fast spiking cells in strata oriens and pyramidale[40].

This inhibitory network model resembles the recurrent inhibitory loop mechanism (above), in that both are sensitive to IPSP decay constants. It differs in that it does not require intact fast EPSPs, and it does require tonic excitation of the interneurones (which is the case experimentally in the hippocampus and at least part of the neocortex). The recurrent inhibition model appears to be stabilized by mutual connections within the population of inhibitory neurones[32] (and also by mutually excitatory neurones[39]), but this is very different from the central role that such connections play in the inhibitory network model.

This new model predicts that both excitatory and inhibitory neurones fire in phase with the gamma rhythm, because both types of neurone are clocked by the same population IPSPs. This is the case in the hippocampus *in vivo*[16], but apparently not in the olfactory bulb and related areas where a phase lag was predicted as a result of the reciprocal activity in the recurrent excitatory–inhibitory loop[5]. Interestingly, the olfactory bulb and anterior olfactory nucleus have a peak in power at oscillation frequencies of around 75 Hz, compared with 40–50 Hz for the hippocampus, and could use different mechanisms. Lateral entorhinal cortex and prepiriform cortex have peaks in power at both these frequencies[5].

Inhibitory network mechanisms might also function in the neocortex. Metabotropic glutamate agonists elicited gamma oscillations when the ionotropic glutamate receptors were blocked pharmacologically, much as they did in the hippocampus[38]. This means that the cortical inhibitory network can sustain gamma oscillations, but we cannot exclude other parallel mechanisms. As mentioned above, the neocortex contains neurones that are intrinsic oscillators at ~40 Hz. We predict that inhibitory neurones that oscillate will tend to stabilize the gamma rhythm of the inhibitory network. At least some intrinsic oscillator neurones are inhibitory.

Golomb, Wang and Rinzel[45] made simulations that show that mutual inhibition can entrain a network, provided that the individual neurones, when uncoupled, oscillate with a short period relative to the inhibitory timecourse. In the case of gamma oscillations in the hippocampus, these conditions are met when τ_{GABA} is in the range 8–13 ms, and there is sufficient tonic 'drive' to the interneurones (Figs 3 and 4). This model was developed for the reticular nucleus of the thalamus, which participates in the generation of synchronous 7–12 Hz 'spindle' discharges and 3 Hz absence-seizure discharges[45,46]. In both cases, the low threshold (T), voltage-dependent Ca^{2+} current plays a crucial role, generating rebound excitation when the IPSPs decay. Computer models showed that full synchronization depends on a sufficiently slow inhibitory potential compared with the excitation component (the low-threshold Ca^{2+} spike)[45–47]. In the case of spindle discharges this is

provided by fast $GABA_A$-mediated IPSPs, and in the case of 3 Hz absence-like seizures, by slow $GABA_B$-mediated IPSPs. In practice, in the diencephalic slice, the thalamic reticular nucleus alone does not produce spindle discharges. Coherent oscillations also require excitatory synaptic input from thalamocortical-projection neurones[45,48]. Without the projection neurones the network tends to generate 'clusters', in which only part of the population participates in the oscillation, which then has a frequency that is an integer multiple (usually $\times 2$ or $\times 3$) of the mean firing rate of individual neurones[45]. This is perhaps easiest to imagine in the case of two mutually inhibitory neurones with rebound excitation; as long as the IPSPs last long enough to deinactivate the T current, then stimulating one will lead to a persistent sequence of alternating discharges in the two neurones. As yet there is no experimental evidence as to whether clustering exists in thalamic tissue.

Inhibitory network synchronization differs between thalamus and hippocampus. In gamma oscillations in the hippocampus, the 'rebound excitation' is not due to a Ca^{2+} current, but rather to the sustained inward current that is turned on by activating mGlu receptors[49]. This causes inhibitory neurones to discharge as soon as the IPSP has decayed sufficiently. More work is needed to find out whether this mechanism applies in other regions that can generate gamma rhythms, such as olfactory structures and neocortex. Some of the mechanisms outlined above could well coexist in particular parts of the brain: inhibitory network and intrinsic mechanisms could combine in the neocortex; and excitatory networks might be essential for the recurrent inhibition loop to work[39]. Our much improved understanding of network oscillators has started to make their mechanisms amenable to direct experimental testing.

Functional consequences of rhythmic inhibition

Both theory[50] and experiment[38,51–53] show that inhibitory neurones are very effective at determining when a pyramidal cell will fire. Our proposal is that the inhibitory network receives a steady or slow excitatory drive which makes it oscillate, providing a clock which determines when pyramidal cells can fire, if they receive suprathreshold, excitatory afferent inputs. The fact that the inhibitory network can, by itself, sustain a rhythm in the gamma frequency range without a requirement for fast EPSPs, separates the synchronizing control or 'clock' from the specific neuronal processing of information 'the Central Processing Unit'. A possible implication is this: if firing of pyramidal neurones is constrained to occur at particular times imposed by a 40 Hz interneuronal network clock, then brain regions expressing 40 Hz might not use rate-encoding of information, but rather might encode information through selection of which pyramidal cells fire at all. Further work also is required to find out whether this provides the means for associating or binding attributes of specific objects, for controlling summation and potentiation, or for a phase code for neural signalling.

If the role of gamma rhythms is indeed to mediate binding, then mechanisms must exist for the selective coupling of areas involved in processing common entities. One idea is that reciprocal excitatory connections can do this, although there seems to be a constraint in that the conduction delay must be less than one third of the period of the rhythm[54]. Others argue that the coherence provided by this kind of mechanism is too weak to provide for reliable binding, in that it appears in simulations only when multiple trials are used[32]. Scaling to larger networks or the presence of intrinsic oscillators, or both, might circumvent this problem. Alternatively (or additionally) oscillations in different parts of the cortex could resonate through the thalamocortical loop[35] (but see Ref. 1). We have a long way to go before we can identify how these long-range links are made and broken, and understanding the mechanisms of local network oscillations is a key step in this direction.

We started this review describing the association of fast rhythms with higher cortical functions. Evidence from MEG recordings suggests that impairments of these rhythms could be involved in brain disease such as Alzheimer's disease[23]. The relationship of gamma rhythms with selective attention has prompted ideas that disruption of its mechanism could play a role in schizophrenia[19]. Understanding the cellular and network mechanisms that generate gamma rhythms provides a starting point for thinking how they could be manipulated therapeutically.

Selected references

1 Gray, C.M. (1994) *J. Comput. Neurosci.* 1, 11–38
2 Engel, A.K. *et al.* (1992) *Trends Neurosci.* 15, 218–226
3 Singer, W. and Gray, C.M. (1995) *Annu. Rev. Neurosci.* 18, 555–586
4 Adrian, E.D. (1950) *Electroencephalogr. Clin. Neurophysiol.* 2, 377–388
5 Eeckman, F.H. and Freeman, W.J. (1990) *Brain Res.* 528, 238–244
6 Gray, C.M. *et al.* (1989) *Nature* 338, 334–337
7 Engel, A.K. *et al.* (1991) *Science* 252, 1177–1179
8 Engel, A.K., König, P. and Singer, W. (1991) *Proc. Natl Acad. Sci. USA* 88, 9136–9140
9 Freeman, W.J. and van Dijk, B.W. (1987) *Brain Res.* 422, 267–276
10 Madler, C. *et al.* (1991) *Br. J. Anaesth.* 66, 81–87
11 Keller, I. *et al.* (1990) *Clin. Electroencephalogr.* 21, 88–92
12 Bouyer, J.J. *et al.* (1987) *Neuroscience* 22, 863–869
13 Pfurtscheller, G., Flotzinger, D. and Neuper, C. (1994) *Electroencephalogr. Clin. Neurophysiol.* 90, 456–460
14 Murthy, V.N. and Fetz, E.E. (1992) *Proc. Natl Acad. Sci. USA* 89, 5670–5674
15 Sanes, J.N. and Donoghue, J.P. (1993) *Proc. Natl Acad. Sci. USA* 90, 4470–4474
16 Bragin, A. *et al.* (1995) *J. Neurosci.* 15, 47–60
17 Stumpf, C. (1965) *Electroencephalogr. Clin. Neurophysiol.* 18, 477–486
18 Soltesz, I. and Deschênes, M. (1993) *J. Neurophysiol.* 70, 97–116
19 Tiitinen, H. *et al.* (1993) *Nature* 364, 59–60
20 Pantev, C. *et al.* (1991) *Proc. Natl Acad. Sci. USA* 88, 8996–9000
21 Kulli, J. and Koch, C. (1991) *Trends Neurosci.* 14, 6–10
22 Galambos, R., Makeig, S. and Talmachoff, P.J. (1981) *Proc. Natl Acad. Sci. USA* 78, 2643–2647
23 Ribary, U. *et al.* (1991) *Proc. Natl Acad. Sci. USA* 88, 11037–11041
24 Llinás, R. and Ribary, U. (1993) *Proc. Natl Acad. Sci. USA* 90, 2078–2081
25 von der Malsburg, C. and Schneider, W. (1986) *Biol. Cybern.* 54, 29–40
26 Gray, C.M. *et al.* (1992) *Visual Neurosci.* 8, 337–347
27 Engel, A.K., König, P. and Schillen, T.B. (1992) *Curr. Biol.* 2, 332–334
28 Young, M.P., Tanaka, K. and Yamane, S. (1992) *J. Neurophysiol.* 67, 1464–1474
29 Tovee, M.J. and Rolls, E.T. (1992) *NeuroReport* 3, 369–372
30 O'Keefe, J. and Recce, M.L. (1993) *Hippocampus* 3, 317–330
31 Hopfield, J.J. (1995) *Nature* 376, 33–36
32 Wilson, M.A. and Bower, J.M. (1991) *Neural Comput.* 3, 498–509
33 Wilson, M. and Bower, J.M. (1992) *J. Neurophysiol.* 67, 981–995
34 Pinault, D. and Deschênes, M. (1992) *Neuroscience* 51, 245–258
35 Steriade, M., Curro Dossi, R. and Contreras, D. (1993) *Neuroscience* 56, 1–9
36 Llinás, R.R., Grace, A.A. and Yarom, Y. (1991) *Proc. Natl Acad. Sci. USA* 88, 897–901
37 Nuñez, A., Amzica, F. and Steriade, M. (1992) *Neuroscience* 51, 7–10
38 Whittington, M.A., Traub, R.D. and Jefferys, J.G.R. (1995) *Nature* 373, 612–615

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39 Ermentrout, G.B. (1995) in *The Handbook of Brain Theory and Neural Networks* (Arbib, M.A., ed.), pp. 732–738, MIT Press
40 Traub, R.D. *et al.* *J. Physiol.* (in press)
41 McCormick, D.A., Gray, C.M. and Wang, Z. (1993) *Soc. Neurosci. Abstr.* 19, 359
42 Gutfreund, Y., Yarom, Y. and Segev, I. (1995) *J. Physiol.* 483, 621–640
43 Wang, X-J. (1993) *NeuroReport* 5, 221–224
44 Llinás, R.R. (1988) *Science* 242, 1654–1664
45 Golomb, D., Wang, X-J. and Rinzel, J. (1994) *J. Neurophysiol.* 72, 1109–1126
46 Wang, X-J. and Rinzel, J. (1993) *Neuroscience* 53, 899–904
47 Destexhe, A. *et al.* (1994) *J. Neurophysiol.* 72, 803–818
48 Von Krosigk, M., Bal, T. and McCormick, D.A. (1993) *Science* 261, 361–364
49 McBain, C.J., DiChiara, T.J. and Kauer, J.A. (1994) *J. Neurosci.* 14, 4433–4445
50 Lytton, W.W. and Sejnowski, T.J. (1991) *J. Neurophysiol.* 66, 1059–1078
51 Buhl, E.H., Halasy, K. and Somogyi, P. (1994) *Nature* 368, 823–828
52 Lacaille, J.C. *et al.* (1987) *J. Neurosci.* 7, 1979–1993
53 Knowles, W.D. and Schwartzkroin, P.A. (1981) *J. Neurosci.* 1, 318–322
54 König, P., Engel, A.K. and Singer, W. (1995) *Proc. Natl Acad. Sci. USA* 92, 290–294

BOOK REVIEW

Methods in Enzymology. Vol. 255 – Small GTPases and Their Regulators, Part A: Ras Family; Vol. 256 – Small GTPases and Their Regulators, Part B: Rho Family

edited by W.E. Balch, C.J. Der and A. Hall, Academic Press, 1995. $99.00 (xxxi + 548 pages) ISBN 0 12 182156 0 (Vol. 255); $80.00 (xxix + 401 pages) ISBN 0 12 182157 9 (Vol. 256)

The Ras and Rho proteins are members of a large superfamily of small GTPases that are activated upon GTP binding and return to an 'off' state when GTP is cleaved to GDP + P_i as a result of their intrinsic GTPase property. The Ras proteins encoded by viral *Ras* genes differ from those encoded by cellular genes by a few amino acids. These point mutations impair their GTPase activity and therefore interfere with their normal shut-off mechanism, making them constitutively active.

The different interconversion states of the GTPases (which has made them popularly known as 'molecular switches') regulate many intracellular signalling pathways. Although both the Ras and Rho family of GTPases belong to a class of proteins that end with the sequence CXXXX, they are functionally distinct. Ras proteins regulate cell growth and differentiation by providing a link between growth factor receptors and gene expression[1], whereas Rho proteins regulate the assembly of focal adhesions and cell movement. Although Rho proteins belong to the GTPase superfamily, they are mainly involved in the regulation of actin cytoskeletal organization[2,3].

Volumes 255 and 256 of Methods in Enzymology are dedicated to the biology and biochemistry of Ras- and Rho-related proteins, respectively, and are divided into four sections. Both volumes are categorized in a similar fashion. The first section describes the methods used for the cloning and purification of recombinant Ras or Rho proteins from bacteria, yeast and the baculovirus–insect-cell system. In this section, emphasis is placed on the post-translational modifications of the Ras proteins, which makes them hydrophobic and allows them to become attached to the plasma membrane. The importance of this modification is well addressed.

The second section deals mainly with the cyclic process of interconversion between the GTP ('on') state and the GDP ('off') state of Ras proteins. The technical details required to monitor the GTP-binding property both at an *in vivo* (*in situ*) level and at an *in vitro* level have been adequately covered.

The third section describes the various approaches taken to identify the protein–protein interactions between components of the Ras-related signal transduction pathway, for which a range of techniques has been widely used, from classical techniques (such as metabolic labeling and immunoprecipitation) to more recent molecular approaches (such as the two-hybrid system).

The final section describes the various fascinating approaches that have been used to monitor the biological activity of *Ras* genes, which include oocyte and mammalian microinjection assays, fibroblast complementation assays and the screening of phage peptide libraries for SH3 ligands.

Both of these volumes have several strengths: the readable and concise collection of chapters are written by some of the leaders in the field of signal transduction, the logical organization of information makes it quick to access information related to specific *Ras* or *Rho* genes, and many of the figures are generally convincing and well done, particularly the photomicrographs in the section 'the role of Rho proteins in cellular function', which are of excellent quality.

However, there are a few areas where these books are not as strong as they might be. The lack of detailed introduction in some of the chapters might make it difficult to follow for students or those entering the area of signal transduction for the first time, and a few articles have not been appropriately assigned to the relevant chapters. In some articles the authors have failed to emphasize the importance of post-translational differences (such as glycosylation, phosphorylation and farnesylation) for the function of Ras-related proteins when expressing them in different systems (for example, *E. coli*, yeast and insect cells). This distinction is important and could have been indicated. The importance of selecting the correct system or cell line has been ignored in the chapters that describe protein–protein interactions, which could lead to bona fide protein–protein interactions being missed due to weak expression or lack of interacting proteins. In addition, misfolding of proteins (for example, in *E. coli*, yeast, Sf9 cells or mammalian cell lines) or failure of certain post-translational events in the target proteins might lead to incorrect conclusions. The antisense approach that has been used to inhibit Ras function is convincing, but control experiments are missing or have not been described here[4].

Overall, these books are a most useful and valuable resource to everyone involved in the field of protein research. They will certainly serve as guidance books and many of the techniques described might remain central to the field of signal transduction in the future.

Tilat A. Rizvi
Dept of Cell Biology, Neurobiology and Anatomy, University of Cincinnati Medical Center, 231 Bethesda Avenue, Cincinnati, OH 45267-0521, USA.

References

1 Barbacid, M. (1987) *Annu. Rev. Biochem.* 56, 779–827
2 Burridge, K. *et al.* (1988) *Annu. Rev. Cell Biol.* 4, 487–525
3 Vincent, S., Jeanteur, P. and Fort, P. (1992) *Mol. Cell. Biol.* 12, 3138–3148
4 Gura, T. (1995) *Science* 270, 575–577

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