breast and ovarian cancer is well established, but the mechanism of tumour suppression by BRCA1 remains to be elucidated. Here we have defined a transcriptional transactivation activity by the C-terminal portion of BRCA1, a result consistent with the reported nuclear localization of BRCA1 (refs 7–9, and H. Ruffner and I. V., unpublished observations). Although some protein sequences fortuitously show transcriptional transactivation activity, the lack of this activity in four different *BRCA1* mutations in families predisposed to breast and ovarian cancer suggests that transactivation may be a true function of BRCA1. If so, we would expect this gene to bind DNA and to regulate a set of target genes. Demonstration of such activities is necessary to confirm our findings and to improve our understanding of the role of *BRCA1* in tumour suppression.

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Insect vibrational defence signals

SIR — A hallmark of insect eusociality is the existence of elaborate communication systems among colony members that permit adaptive responses to changes in the environment[1]. I describe an analogous communication system in a subsocial insect, the treehopper *Umbonia crassicornis* (Insecta: Homoptera: Membracidae). In this species, females provide care for aggregations of nymphal offspring[2]. Aggregations extend for up to 15 cm along the host-plant stem and contain up to 80 individuals. When attacked by a predator, nymphs produce substrate-borne vibrational signals that rapidly elicit their mother's antipredator behaviour. Signals produced by nymphs at the site of the attack prompt signalling by their siblings. The result is a composite, group signal, and only group signals evoke the mother's antipredator response.

Nymphal *U. crassicornis* are preyed upon by a range of invertebrates[3,4]. Their most important predators are wasps, which include treehopper specialists[5,6]. Maternal protection, including approaching the predator, wing fanning and kicking with club-shaped hind legs[3,6], is the nymphs' only defence against attack by invertebrate predators.

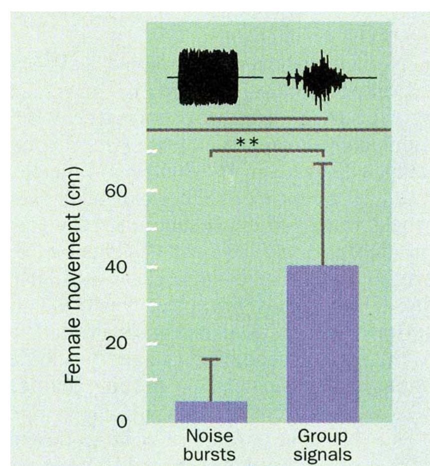
The vibrational signal produced by a nymph is a brief series of pulses lasting 30–40 ms. After one individual is attacked by a predator, signalling travels in a wave through the aggregation. Individual signals thereby combine to form a composite, group signal with a duration of 300–600 ms. Disturbed aggregations produce group signals at intervals of 1–2 s.

Females respond to vibrational playback of group signals, but not simply to any vibrational stimulus from the aggregation site. Females rapidly approached the vibration source in response to playback of group signals from their offspring (removed from the plant during playbacks), occasionally kicking and wing fanning. In contrast, most females remained stationary (as during prestimulus periods) in response to noise bursts of the same duration, repetition rate, and peak amplitude (see figure).

Coordination among signalling nymphs is necessary to evoke the female's response. Playback of 10 individual offspring signals digitally combined into the coordinated pattern of natural group signals caused females rapidly to approach the signal source, as in the previous experiment. However, females showed little or no response to the same signals arranged in random temporal patterns (Wilcoxon signed-rank test, $n=12$, $P<0.01$).

How is this coordination achieved, given that only one or a few nymphs may be contacted directly by a predator? Playback of group signals (with the female removed from the plant) consistently elicited signalling from otherwise undisturbed nymphs, while playback of noise bursts of the same duration, peak amplitude and repetition rate did not (Wilcoxon signed-rank test, $n=15$ aggregations, $P<0.01$). Even when they are not immediately threatened, nymphs will thus signal in response to signals from siblings.

During field observations of the closely related species *U. spinosa,* group signalling occurred almost exclusively in the presence of predators. In 7 days of observation of 10 aggregations near Gamboa, Panama, I observed 60 predation attempts on nymphs by vespid wasps (primarily *Pseudopolybia compressa*). Undisturbed nymphs were silent or produced sporadic individual signals, but nymphs attacked by wasps produced a rapid series of vibrational group signals. Females successfully defended nymphal aggregations in 54 out of 60 attacks (90%), and in only 6 cases (10%) were



Responses of female *U. crassicornis* to vibrational playback (representative playback stimuli are shown in upper panel, where scale bar represents 1 s). In response to group signals, previously stationary females rapidly approached the area where the aggregation had been, then engaged in apparent searching behaviour; in response to noise bursts (which differed from natural signals in the time and frequency domains), most females remained stationary (Wilcoxon signed-rank test, $n=14$, $P < 0.01$). The measure shown represents distance moved by females during a 3-min stimulus period. Signals contained energy mainly from 100–4,000 Hz and were transduced with a lightweight accelerometer (0.28 g; frequency response flat from 20–5,500 Hz) glued to the woody host-plant stem. Stimuli were played from a MacIntosh IIsi computer and coupled to the host-plant stem using an electrodynamic shaker (Labworks ET-203). As found in other studies[7], the resulting signals propagated in the plant stem were bending waves; this was also true of the natural signals (my unpublished data). The vibrational playback system used a closed-loop procedure to ensure that playback signals matched natural signals in amplitude and frequency characteristics. Shaker output was monitored with an accelerometer at the site of the playback subject to calculate the filtering characteristics of the entire system. Inverted filter coefficients were derived to compensate for this filtering (which was largely due to the plant transmission channel), and these were applied to the playback stimuli. As a result, when a natural signal was played back, it closely matched the frequency spectrum of that same signal when first recorded at that site (amplitude spectra equal to within 0.5 dB in 164-Hz bins up to 5.5 kHz). Stimulus pairs were adjusted to be equal in peak amplitude to natural signals, and to each other, at the site of the playback subject. The order of stimulus presentation was alternated between subjects in each experiment.

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wasps successful in removing a nymph. In contrast, the success rate of predatory wasps can be as high as 90% in the absence of tending females[6]. Offspring–parent signalling appears to play a central role in defence in these subsocial insects. As in eusocial taxa, communication among group members permits an adaptive response to a rapidly changing feature of the environment.

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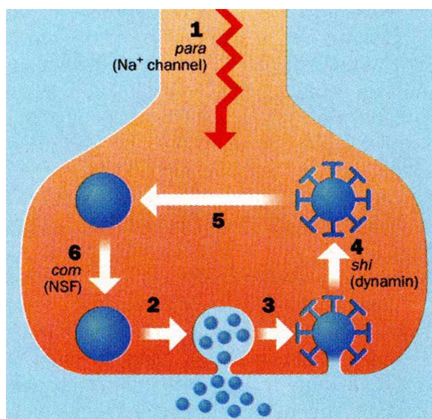
Classic clues to NSF function

SIR — Most intracellular membrane fusion events require the action of the *N*-ethylmaleimide-sensitive fusion protein (NSF)[1]. The role of NSF in vesicular transport remains highly controversial, and various models have proposed that NSF acts at a post-docking stage close to membrane fusion[2], at a post-docking but pre-fusion stage[3], or at a pre-docking stage[4,5]. A recent study of *in vitro* vacuolar fusion in yeast[6] supports the latter hypothesis, as does re-examination of a 1976 report on the temperature-sensitive *Drosophila* mutant *comatose*[7], which is now known to be an NSF mutant[8].

Like many other *in vitro* membrane fusion assays[1], homotypic fusion of yeast vacuolar vesicles requires NSF (Sec18p in yeast). Using an elegant approach, Mayer *et al.*[6] have succeeded in kinetically defining the stage of action of NSF in the complex process of priming, docking and fusion of vesicles that comprises the vacuolar fusion assay. They found that the action of Sec18p is complete even before docking can occur, implying that NSF acts to prime vesicles for subsequent docking and/or fusion.



Neurotransmission and synaptic vesicle recycling in *Drosophila* synapses. Stages depicted: 1, action potential propogation; 2, synaptic vesicle exocytosis and neurotransmitter release; 3, coated pit formation; 4, endocytic vesicle formation; 5, vesicle uncoating and reloading with neurotransmitter; 6, priming of vesicle for docking and/or fusion. The putative stages at which paralytic mutants are blocked in this cycle are illustrated.

Although the literature is replete with data on NSF function in constitutive membrane traffic[1] such as the vacuolar fusion assay, the only functional evidence for a role of NSF in regulated membrane traffic, as reported in *Nature*[8], is that the temperature-sensitive paralysis exhibited by *comatose* mutants of *Drosophila* are due to point mutations in the *dNSF-1* gene. The original, classic work on *comatose* by Siddiqi and Benzer[7], when reinterpreted 20 years later, provides important information on the molecular function of NSF in neurotransmission *in vivo*.

Siddiqi and Benzer[7] observed the kinetics of onset and recovery from temperature-induced paralysis in three *Drosophila* mutants: *para* (*paralysed*), *shi* (*shibire*) and *com* (*comatose*), all of which result from a presynaptic block of neurotransmission. *Para* mutants became paralysed within seconds of a temperature shift and recovered almost instantaneously, whereas *com* mutants required a minute to become fully paralysed and 30 minutes to recover; *shi* mutants were intermediate, displaying complete paralysis at 30 seconds and recovering after 20 minutes. Although phenomenological at the time, these data have profound functional significance as it is now known that the *para* gene codes for a voltage-dependent sodium channel[9], the *shi* gene for dynamin[10] and the *com* gene for NSF[8].

Neurotransmission begins with an action potential and results in the release by exocytosis of neurotransmitter, which then signals to the postsynaptic cell (stages 1 and 2 in the figure). Nevertheless, this complex process can take place in less than 200 microseconds. However, the depletion of synaptic vesicles by exocytosis has to be balanced by replenishment of new synaptic vesicles via endocytosis in a process estimated to require many seconds[11] (stages 3–6 in the figure). The kinetics of temperature-induced paralysis fit this model, as *para* flies exhibiting defects in voltage-dependent Na^+ channels (required to generate an action potential) recover from paralysis almost instantaneously, as would be predicted if an essential switch for neurotransmission was suddenly turned on. Indeed, such fast kinetics are exhibited not only by different allelic mutants of *para*, but also by *nap* (*no action potential*) and *tip-E* (*temperature-induced paralysis-E*) *Drosophila* mutants, which have different Na^+ channel defects[12]. In contrast, *shi* flies exhibiting mutations in dynamin (required for endocytic vesicle formation) take 20 minutes to recover fully from paralysis, as would be predicted if an essential switch for synaptic vesicle replenishment was suddenly turned on (the synaptic vesicle pool in *shi* mutants is replenished after 15 minutes[13]).

As the time taken for *com* flies to recover from paralysis is even longer than that required for *shi* flies and as the known allelic mutants of *com* display similar kinetics of paralysis, this suggests that the action of NSF is slower than the process of synaptic vesicle recycling. The new work on vacuolar fusion[6] suggests that this action is the priming of vesicles for cell membrane docking and fusion. Synaptic vesicle recycling is thought to involve two processes, endocytosis and vesicle re-priming, both requiring many seconds[11]. The slow kinetics of *shi* and *com* mutants[7] support a model in which dynamin acts in endocytosis and NSF in re-priming recycled synaptic vesicles (see figure). Such a slow, priming action of NSF is consistent with the hypothesis that NSF acts as a molecular chaperone to fold *Botulinum* neurotoxin substrates into a conformation competent for vesicle docking and/or fusion[4]. In addition to the *Drosophila* mutants discussed above, there are many temperature-sensitive paralytic mutants with differing kinetics whose mutant genes are unknown[12]. On identification of these genes, a rich source of data from previous decades should lead to insights on their *in vivo* molecular function in synaptic vesicle dynamics based on the kinetics of their paralytic phenotypes.

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Scientific Correspondence

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